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Mechanisms for Insulin-dependent Regulation of Skeletal Muscle Mitochondria by OPA-1

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MECHANISMS FOR INSULIN-DEPENDENT REGULATION OF SKELETAL MUSCLE MITOCHONDRIA
BY OPA-1

by

Serif Bacevac

A thesis submitted in partial fulfillment of the requirements
for graduation with Honors in the Health and Human Physiology

E. Dale Abel; Antentor Hinton
Thesis Mentor

Spring 2018

All requirements for graduation with Honors in the
Health and Human Physiology have been completed.

Gary L. Pierce
Health and Human Physiology Honors Advisor

Mechanisms for insulin-dependent regulation of skeletal muscle mitochondria by OPA1.

A Thesis Submitted to the Faculties of

The Human Physiology Honor's Program

University of Iowa

In Fulfillment of the

Requirement for Honor's in The Major

By

Serif Bacevac

Iowa city, Iowa

May 9, 2018

APPROVED BY THE HONOR (THESIS) COMMITTEE

Signed _____

Dr. E. Dale Abel, Primary Mentor

Dr. Antentor O. Hinton, Secondary Mentor

Dr. Gary Pierce, Honor's Advisor

Dr. Vitor Lira, Honors Committee Member

Dr. Melissa Bates, Honor's Committee Member

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II. Abstract:

Augmentation of mitochondrial oxidative metabolism by insulin might be mediated in part by induction of the inner mitochondrial membrane protein, Optic Atrophy-1 (OPA-1). Decreased OPA-1 in skeletal muscle has been reported in both murine and human type II diabetic models. Reduced OPA-1 in these contexts correlates with mitochondrial content, oxidative capacity, and dynamics. In

addition to increasing mitochondrial fusion, OPA-1 mediates cristae remodeling to enhance mitochondrial bioenergetics through oligomer formation. Therefore, we hypothesized that insulin stimulation increases OPA-1 protein levels and mitochondrial respiration by increasing OPA-1 oligomerization and cristae remodeling in skeletal muscle cells. To investigate the role of insulin on mitochondrial dynamics and cristae organization, Cre-LoxP technology was used to ablate OPA-1 from skeletal myoblasts isolated from floxed OPA-1 mice. Confocal microscopy and MitoTracker orange was employed to obtain three-dimensional images of mitochondria networks, and transmission electron microscopy (TEM) was used to analyze ultrastructure of mitochondrial cristae. Two hours of insulin treatment increased OPA-1 protein levels, promoted mitochondrial fusion, elevated mitochondrial respiration, increased cristae number along with volume density and area, decreased the distance of ER-mitochondria contacts, and it may have even changed OPA-1 oligomerization. Depletion of OPA-1 and MFN-2 blocked the metabolic effects of insulin stimulation. OPA-1 depletion reduced cristae number, volume density, and area. Loss of OPA-1 resulted in a compensatory elevation of mitochondrial associated membrane proteins (MAMs), increasing the number of ER-mitochondria contacts, blocking OPA-1 oligomerization, and decreasing the ER-mitochondria contact distance. Together, ablation of OPA-1 alters metabolic responses to insulin in skeletal muscle cells. Furthermore, insulin stimulation promotes changes in cristae morphology and triggers mitochondrial fusion through an IR-pAKT pathway that increases OPA-1. ER-mitochondria contacts increase after ablation of OPA-1 due to an increase in MAMs. Lastly, Loss of OPA-1 inhibits OPA-1 oligomerization formation.

III. Introduction:

Background and Purpose: Diabetes and cardiovascular disease (CVD). Diabetes affects 29.1 million people in the United States, and another 86 million have prediabetes (26). Although 1 out of 11 people has diabetes, 1 out of 4 people are unaware (26). Diabetes disproportionately affects minorities, especially Native Americans and African Americans (26). Type 2 diabetes (T2DM) has been linked to alterations in mitochondrial oxidative metabolism in insulin responsive tissues, characterized by reduced mitochondrial content, and acquired or reduced submaximal ADP-stimulated oxidative phosphorylation and plasticity

of mitochondria. T2DM is strongly related to insulin resistance (6), which is reduced responses in target tissues, such as skeletal muscle or liver (6). Insulin, produced by the pancreas, is a hormone that helps use energy from blood glucose. (6). Although β -cell failure is the *condicio sine qua non* of T2DM, skeletal muscle insulin resistance often is an early defect before hyperglycemia develops and β -cell failure occurs (6; 15; 32). Notably, muscle lipid accumulation is associated with insulin resistance and T2DM in children and adults (9). High blood glucose in adults with diabetes increases risk for stroke and manifestations of coronary

artery disease such as angina, and heart attack (10; 12). People with T2DM have increased prevalence of high blood pressure, dyslipidemia, and obesity that independently contribute to high rates of CVD (1; 10; 12), which is the leading cause of death of people with diabetes (1; 4; 10). Although some interventions may increase oxidative phosphorylation, mitochondrial content and improve insulin resistance in some subjects (11), however, important gaps remain in our understanding of the link between mitochondrial dysfunction and insulin resistance.

Mitochondrial Dynamics. Mitochondria are exceptional double membrane organelles (24). They act as cellular powerhouses producing large quantities of energy to maintain normal homeostasis. Mitochondria contain two membranes for specific functions—the outer membrane, which has pores to allow ions and small molecules to flow through and the inner membrane that houses the mitochondrial genome and the electron transport chain (18; 23; 24). These two mitochondrial membranes are dynamic and undergo constant cycling to maintain mitochondrial integrity, by mechanisms called fusion and fission (14; 18; 23; 24). Mitochondrial fission and fusion play critical roles in protecting the mitochondria under diverse metabolic or environmental stresses (22). Fusion mitigates stress by amalgamating damaged mitochondria as a form of complementation whereas fission creates new mitochondria and functions to remove damaged mitochondria via mitophagy or to activate apoptosis (5; 35). Mitochondrial proteins that regulate outer membrane fusion belong to a GTPase family called Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2); whereas, Optic atrophy 1 (OPA1) controls the inner mitochondrial membrane fusion (7; 19; 30). Fusion of each membrane is distinct (14; 18; 20; 23; 24). Interestingly, the inner membrane regulates mitochondrial cristae morphology in part by generation of OPA1 oligomers (7; 19; 22). These oligomers of OPA1 are assembled

at the base of the mitochondrial cristae to narrow the width (7; 19; 22). Conversely, mitochondrial fission is mediated by interactions between two proteins, Mitochondrial fission 1 protein (Fis1), found in the outer membrane and a cytosolic protein, Dynamin-1-like protein (Drp1) (14; 18; 22; 23; 30). For fission to occur, Drp1 translocates to the mitochondria. Post-translational modifications also regulate Drp1, and these include phosphorylation, ubiquitination, or sumoylation (27; 31; 33). Although mitochondrial dynamics uses different proteins, germline, conditional, and specific tissue mutations show that altered mitochondrial fusion or fission proteins are deleterious for mitochondrial homeostasis suggesting these proteins are essential for maintaining healthy mitochondria.

OPA1 and Insulin Signaling. Mitochondria are central to insulin action in skeletal muscle. Importantly, skeletal muscle mitochondrial dysfunction may lead to fat accumulation in the skeletal muscle, which is implicated in the initial stages of the development of insulin resistance. We reported that insulin increased mitochondrial fusion and oxidative capacity in vivo and in-vitro in skeletal muscle. Increased mitochondrial connectivity is associated with increased crista density. Mechanistically, insulin increases OPA1 mRNA and protein via AKT-mTOR-NFKB transcriptional regulation of the OPA1 promoter (21). Notably, silencing OPA1 or Mfn1 in vitro prevented insulin-mediated stimulation of mitochondrial metabolism. OPA1 oligomerization improves efficiency of the electron transport chain and activity of ATP synthase (21). Oligomerization of OPA1 is independent of its capability to promote mitochondrial fusion (21). Mutations in OPA1, which lack the GTPase domain, maintain oligomerization, which is sufficient to increase efficiency of hepatic mitochondria after short-term nutrient deprivation (22). However, it is unknown if insulin signaling increases OPA1 oligomerization, and whether this mechanism contributes to OPA1-dependent activation of mitochondrial function following insulin stimulation.

IV. Materials and methods:

C2C12 and Human Cell Culture:

Mouse skeletal muscle C2C12 cells were maintained in HG DMEM containing 4.5 g/l glucose (Lonza, Basel, Switzerland) enhanced with 10% heat inactivated Fetal Bovine Serum (FBS) (Gibco/Life Technologies, Carlsbad, CA, USA), 100 µg/ml penicillin and 100 µg/ml streptomycin (Lonza) (growth medium) to maintain

myoblasts Differentiation was started when cells reached ~70–80% confluence (day 0). Differentiation was induced by switching from growth medium to HG DMEM containing 4.5 g/l glucose supplemented with 2% Horse Serum (Gibco/Life Technologies), 100 µg/ml penicillin and 100 µg/ml streptomycin (differentiation medium) to form myotubes.

Primary Cell culture:

Satellite cell isolation was performed as previously described [11].

Western blotting:

To obtain total protein extracts from differentiated myotubes, cells were washed with ice-cold PBS before adding cold lysis buffer (25 mM Tris HCL pH = 7.9), 5 mM MgCl₂, 10% glycerol, 100 mM KCl; 1% NP40; 0.3 mM dithiothreitol, 5 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 50 mM sodium fluoride, containing freshly added protease inhibitor cocktail (Roche Applied Science, Penzberg, Germany). Cells were scraped, homogenized with a 25-gauge needle, and centrifuged at 14,000 rpm for 10 min at 4 °C. Supernatants were collected and boiled with Laemmli sample buffer. Cell lysates were subjected to SDS-Page and proteins were transferred to nitrocellulose membranes (BioRad, Berkeley, California, USA). Membranes were blocked with 3% BSA-TBST and incubated with anti-AMPK α , anti-phospho-AMPK α , anti-GRP 75, anti-PACS2, anti-OPA1, anti-fis1, anti-MFN1, anti-MFN2, anti-Drp1, anti-Drp1-673, anti-Drp1-616, and anti-Actin. Quantification was carried out using ImageJ 1.49 m. For statistical analysis Student T-tests were used.

Antibodies:

Primary Antibodies: OPA1 (1:1,000, BD Biosciences, San Jose, CA, USA, #612606), FGF21 (1: 1,000, Abcam, Cambridge, UK #ab171941), GAPDH (1: 1,000, Cell Signaling Technology, Danvers, MA, USA, #2118), AMPK (1: 1,000, Cell Signaling Technology #2793), phospho-AMPK (1: 1,000, Cell Signaling Technology #4181), Mfn1 (1: 1,000, Abcam #ab57602), Mfn2 (1: 1,000, Abcam # ab101055), GRP 75, Drp1, Drp 673, Drp 616. Secondary antibodies: IRDye 800CW anti-mouse (1:10,000, LI-COR, Lincoln, NE,

USA, #925- 32212) and Alexa Fluor anti-rabbit 680 (1: 10,000, Invitrogen #A27042). Fluorescence was quantified using the LiCor Odyssey imager.

V. Results:

Figure 1: Cell Line Development

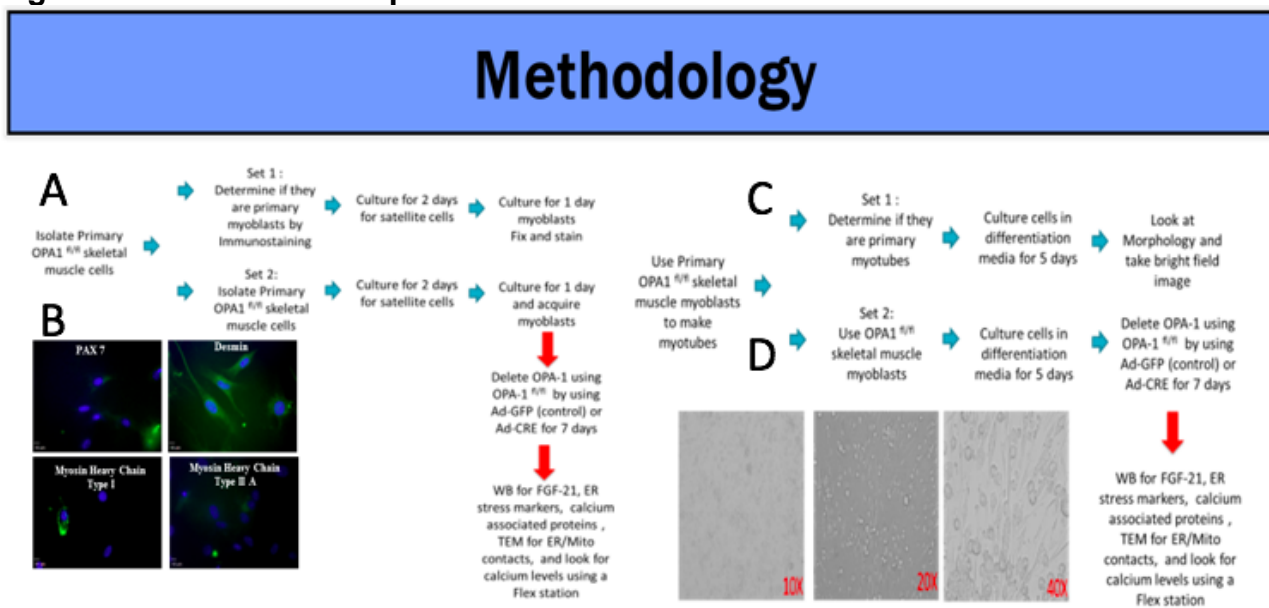


Figure 2: Insulin Stimulation Increases OPA-1 in C2C12 Myoblasts

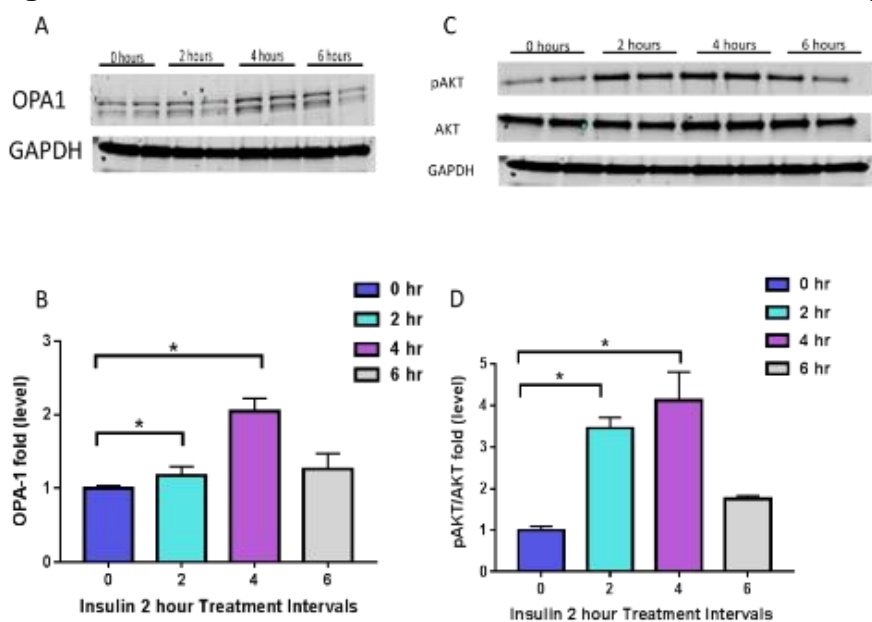


Figure 2A. Representative Western blot image of OPA-1 expression in C2C12 myoblasts after insulin treatment. **Figure 2B.** Quantification of OPA-1 expression in C2C12 myoblasts after insulin treatment. **Figure 2C.** Representative Western blot image of pAKT/tAKT ratio in C2C12 myoblasts after insulin treatment. **Figure 2D.** Quantification of pAKT/tAKT ratio in C2C12 myoblasts after insulin treatment.

Figure 3: Insulin Stimulation Increases OPA1 in C2C12 Myotubes

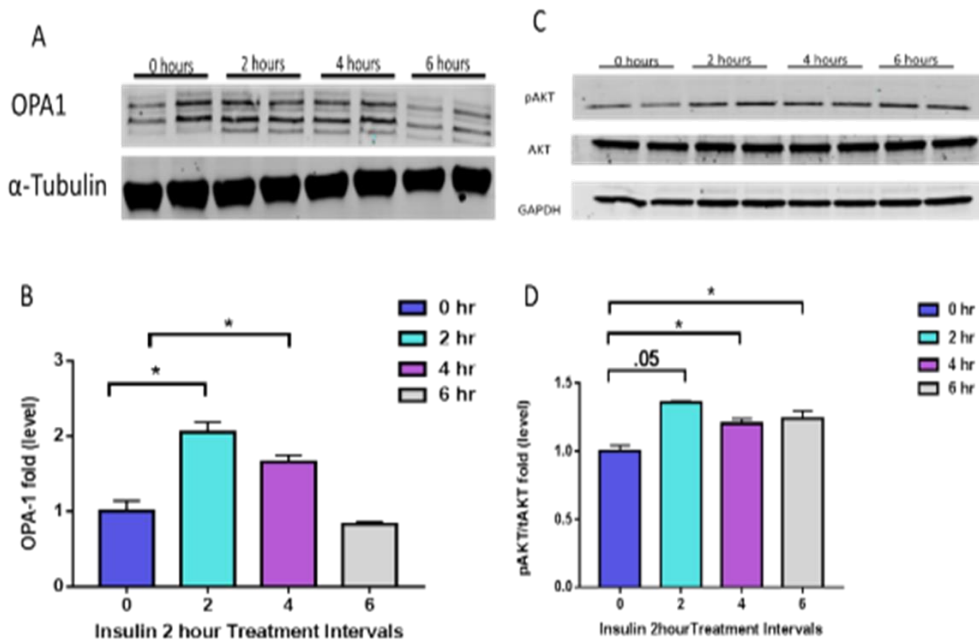


Figure 3A. Representative Western blot image of OPA-1 expression in C2C12 myotubes after insulin treatment. **Figure 3B.** Quantification of OPA-1 expression in C2C12 myotubes after insulin treatment. **Figure 3C.** Representative Western blot image of pAKT/tAKT ratio in C2C12 myotubes after insulin treatment. **Figure 3D.** Quantification of pAKT/tAKT ratio in C2C12 myotubes after insulin treatment.

Insulin increases the levels of Mitochondrial OPA-1 protein in C2C12 myoblasts and C2C12 myotubes: We previously published that insulin increases OPA1 levels and mitochondrial function in neonatal ventricular myocytes and L6 myoblasts [1], in parallel with increased mitochondrial fusion. C2C12 myoblasts were plated in 10% FBS DMEM, and switched 48 hours later to a low-serum medium (2% FBS DMEM) for 12 hours. Myotubes were differentiated in 2% horse serum. Cells were then treated with insulin (10nM/L) for up to 6 hours and analyzed at two-hour intervals. Cells were collected for Western blot analysis. Western blot for OPA-1 demonstrated that insulin treatment increases OPA-1 protein levels over the 6-hour time course in C2C12 myoblasts (Figure 2A and Figure 2B) and C2C12 myotubes (Figure 3A and Figure 3C). Insulin treatment also increased phosphorylation of AKT at Ser 473 in C2C12 myoblasts (Figure 2C and Figure 2D) and C2C12 myotubes (Figure 3C and Figure 3D).

Figure 4: Insulin Stimulation Increases OPA1 in Primary Myoblasts

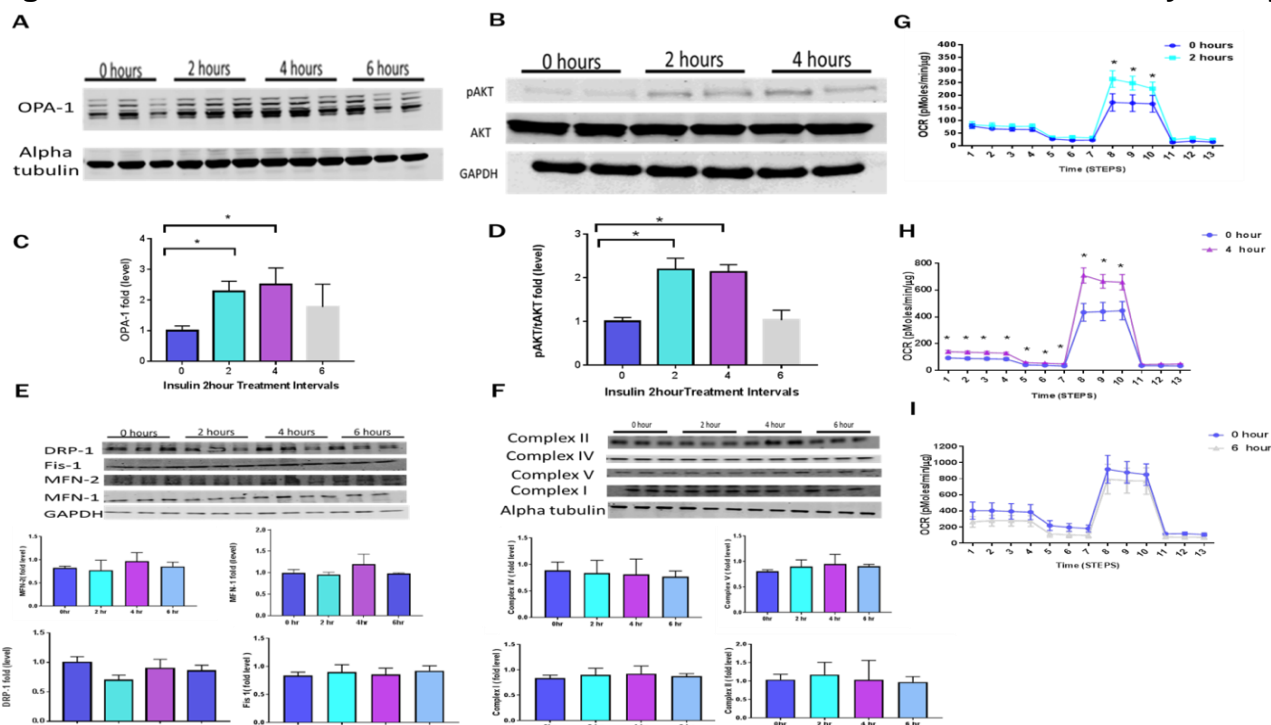
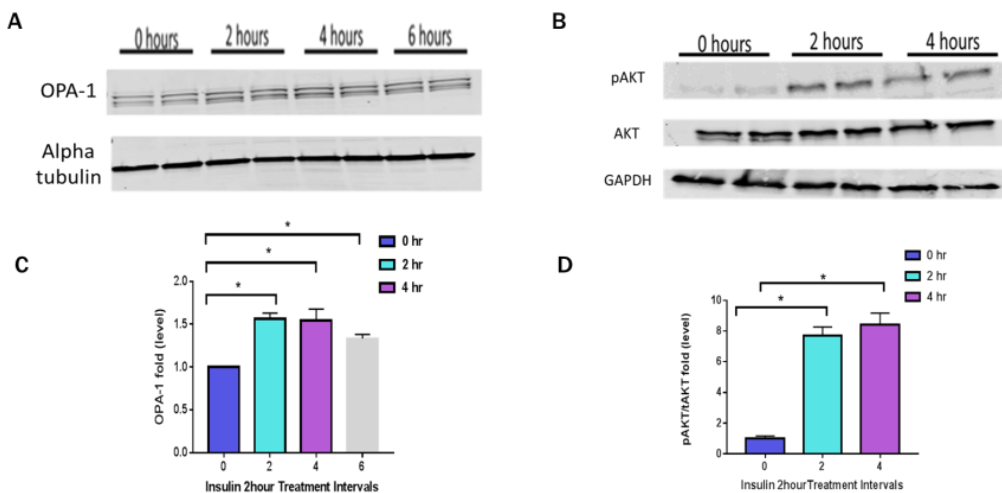


Figure 4A.

Representative Western blot image of OPA-1 expression after insulin treatment in myoblasts. **Figure 4B.** Representative Western blot image of pAKT/tAKT ratio after insulin treatment in myoblasts. **Figure 4C.** Quantification of OPA-1 expression after insulin treatment in myoblasts. **Figure 4D.** Quantification of pAKT/tAKT ratio after insulin stimulation in myoblasts. **Figure 4E.** Western blot image and Quantification of Fusion and Fission proteins after insulin stimulation in myoblasts. **Figure 4F.** Western blot image and Quantification of OXPHOS proteins after insulin stimulation in myoblasts. **Figure 4G-4I.** Seahorse OCR with and without insulin stimulation for 2 hours, **(G)** 4 hours **(H)**, and 6 hours **(I)** in myoblasts.

Figure 5: Insulin Stimulation Increases OPA1 in C2C12 Myotubes



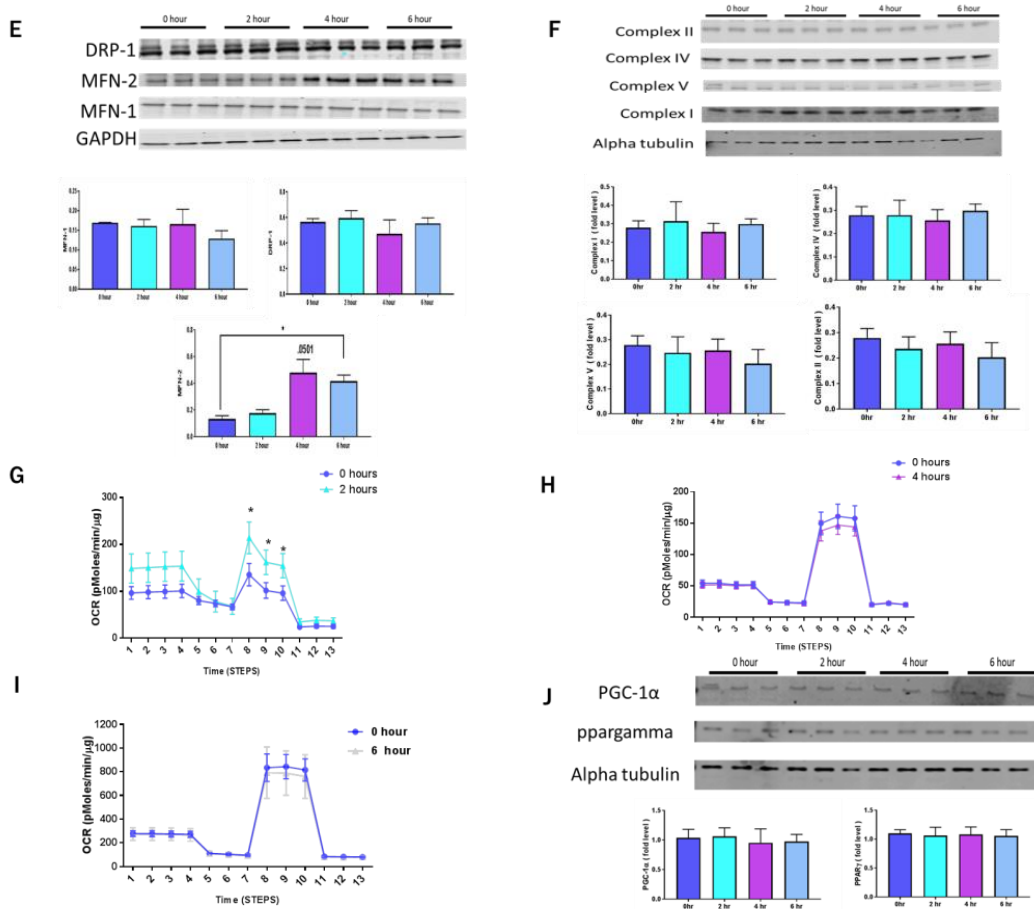


Figure 5A. Representative Western blot image of OPA-1 expression after insulin treatment in myotubes. **Figure 5B.** Representative Western blot image of pAKT/tAKT ratio after insulin treatment in myotubes. **Figure 5C.** Quantification of OPA-1 expression after insulin treatment in myotubes. **Figure 5D.** Quantification of pAKT/tAKT ratio after insulin stimulation in myotubes. **Figure 5E.** Western blot image and Quantification of Fusion and Fission proteins after insulin stimulation in myotubes. **Figure 5F.** Western blot image and Quantification of OXPHOS proteins after insulin stimulation in myotubes. **Figure 5G-5I.** Seahorse OCR with and without insulin stimulation for 2 hours, (G) 4 hours (H), and 6 hours (I) in myotubes. **Figure 5J.** Western blot image and Quantification of Mitochondrial Biogenesis Markers in myotubes.

Insulin Increases Levels of Mitochondrial OPA-1 Protein in Primary Myoblasts and Primary Myotubes without Changing Mitochondrial Biogenesis and Increases Mitochondrial Oxygen Consumption:

To investigate the role of insulin stimulation on OPA-1, we utilized a *primary in vitro system*. Isolation of primary satellite cells were from OPA-1 male floxed mice (Figure 1). Subsequently, cells were plated in 10% FBS DMEM, and switched 48 hours later to a low-serum medium (2% FBS DMEM) for 12 hours. Myotubes were differentiated in 2% FBS serum and insulin transferrin selenium. Primary myoblasts were then validated using either immunocytochemistry markers of skeletal muscle (Figure 1A and Figure 1B) and primary myotubes were validated by bright field morphology (Figure 1C or Figure 1D). Cells were then treated with insulin (10nmol/L) for up to 6 hours and analyzed at two-hour intervals to elucidate the mechanism of insulin dependent mitochondrial fusion by OPA-1. Western blot analysis showed that insulin stimulation increased total content of OPA-1 protein levels at 2 and 4 hours (Figure 4A and Figure 4C) in primary myoblasts and primary myotubes (Figure 5A and Figure 5C). As a positive control, cell lysate was used to determine if the insulin stimulation worked in primary myoblast (Figure 4B and Figure 4D) and primary myoblasts (Figure 5B and Figure 5D) by measuring the activation of AKT at site Ser 473. Insulin stimulation increased AKT at site

Ser 473 at 2 hours and 4 hours of insulin stimulation.

Notably, insulin stimulation did not change MFN-1, DRP-1, FIS-1, and MFN-2 protein expression in primary myoblasts (Figure 4E) and primary myotubes (Figure 5E). To determine whether the changes observed in OPA-1 occurred in conjunction with an increase in mitochondrial biogenesis, we evaluated changes in total mitochondrial mass by quantifying three core proteins of the OXPHOS machinery. Insulin did not change the level of any of these components (Figure 4F and Figure 5F). An increase in OPA-1 protein level has been associated with improved mitochondrial capacity and associated with mitochondrial fusion. To assess mitochondrial capacity, we used searhorseXF-24 to quantify the changes of insulin stimulation on mitochondrial OXPHOS. Insulin promoted a rise in both baseline and FCCP- uncoupled maximum cellular oxygen consumption after 2 hours and 4 hours of insulin treatment in primary myoblasts (Figure 4G and Figure 4H) and at 2 hours in primary myotubes (Figure 5G and Figure 5H). However, prolonged stimulation of insulin (6 hours) resulted in no oxygen consumption changes (Figure 4I and Figure 5I). Although, OXPHOS machinery did not change, we further investigated if mitochondrial biogenesis was increased to account for the changes in OPA-1 protein expression. Western blot analysis demonstrated that PGC1 α , a master regulator of mitochondrial biogenesis did not change in myotubes (Figure 5J).

Figure 6: OPA1 Deletion Decreases Mitochondrial Capacity in Primary Myoblasts and Myotubes

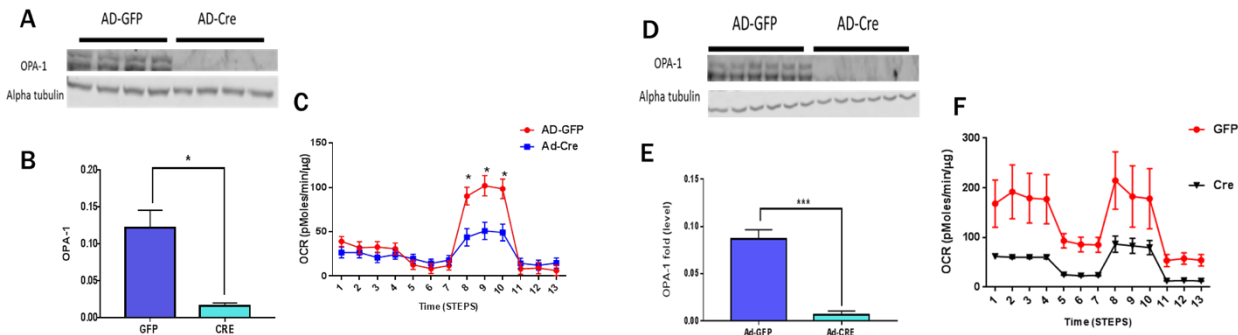


Figure 6A.

Representative Western blot image of OPA-1 deletion in Primary OPA-1 floxed myoblasts **Figure 6B**. Quantification of Western blot image of OPA-1 deletion in Primary OPA-1 floxed myoblasts. **Figure 6C**. Seahorse measurement of mitochondrial respiration (OCR) after Primary deletion of OPA-1 in myoblasts. **Figure 6D**. Representative Western blot image of OPA-1 deletion in Primary OPA-1 floxed myotubes. **Figure 6E**. Quantification of Western blot image of OPA-1 deletion in Primary OPA-1 floxed myotubes. **Figure 6F**. Seahorse measurement of mitochondrial respiration (OCR) after Primary deletion of OPA-1 in myotubes.

Figure 7: Downregulation of OPA1 alters the metabolic response to insulin in primary myotubes

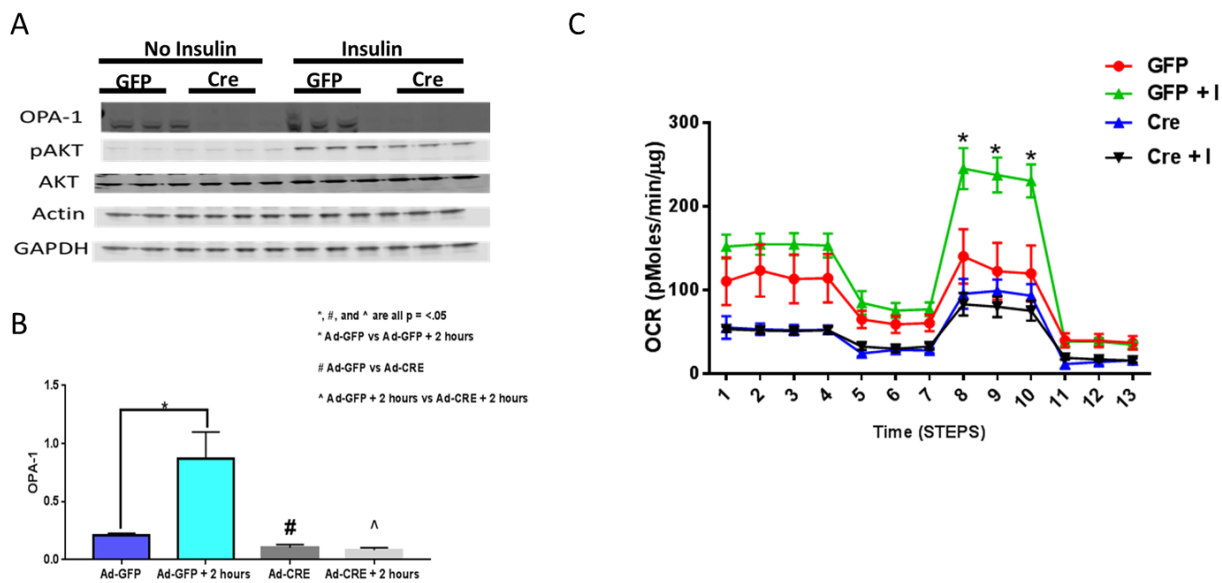


Figure 7A.

Representative Western blot image of OPA-1 deletion in Primary OPA-1 floxed myotubes that have been stimulated with and without insulin. **Figure 7B.** Quantification of Western blot image of OPA-1 deletion in Primary OPA-1 floxed myotubes that have been stimulated with and without insulin. **Figure 7C.** Seahorse measurement of mitochondrial respiration (OCR) after Primary deletion of OPA-1 in myotubes and stimulation with insulin.

Downregulation of OPA-1 Alters the Metabolic Responses to Insulin in Primary Myotubes: To determine if mitochondrial fusion changes are directly related to the effects of insulin on mitochondrial function, we made primary satellite cells from male OPA-1 floxed mice and cells were grown to produce myoblasts in (20% FBS) or differentiated into primary myotubes (2%). After the two primary cell lines were established, cells were infected with a GFP-expressing adenovirus (AD-GFP) or with an adenovirus expressing a GFP-tagged CRE recombinase. Ablation of OPA-1 in primary myoblast was confirmed by Western blot (Figure 6A and Figure 6B). After 48 hours deletion, oxygen consumption was measured by SeahorseXF-24 and we determined that loss of OPA-1 lowered the ability for the cells to respire (Figure 6C). Western blot analysis determined that OPA-1 was ablated in primary myotubes (Figure 6D and Figure 6E) and showed similar oxygen consumption results as primary myotubes (Figure 6F). Short-term deletion of OPA-1 blocked 2-hour insulin mediated increase in oxygen consumption (Figure 7C). Together, without OPA-1 the metabolic responses of insulin are altered in primary myotubes.

Figure 8: Insulin Stimulation Promotes Fusion in the Absence of OPA1

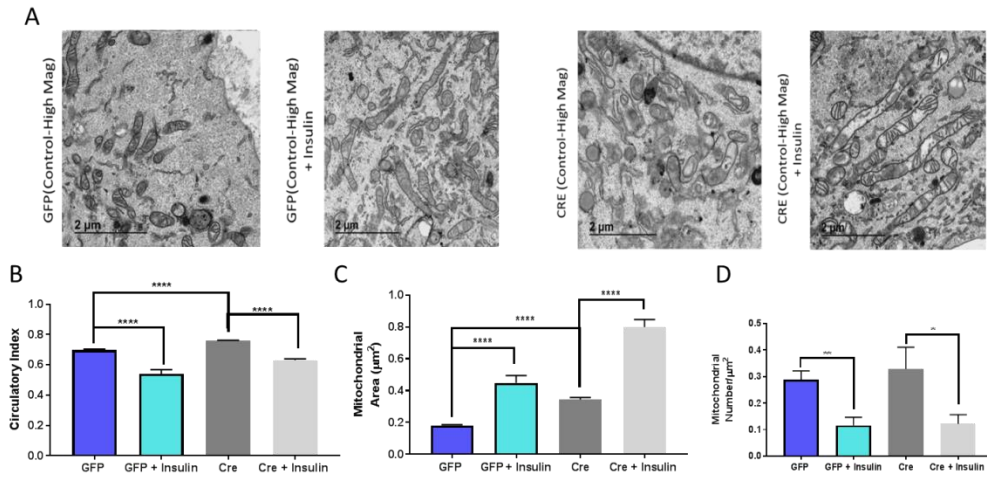


Figure 8A. Representative TEM image of primary myoblasts that received deletion of OPA-1 with or without insulin. **Figure 8B- Figure 8D.** Circulatory Index (**8B**), Mitochondrial Area (**8C**), and Mitochondrial Number (**8D**) of primary myoblasts that received with or without insulin.

Figure 10: Ablation of IR/IGF-R blocks insulin mediated fusion in Myoblasts

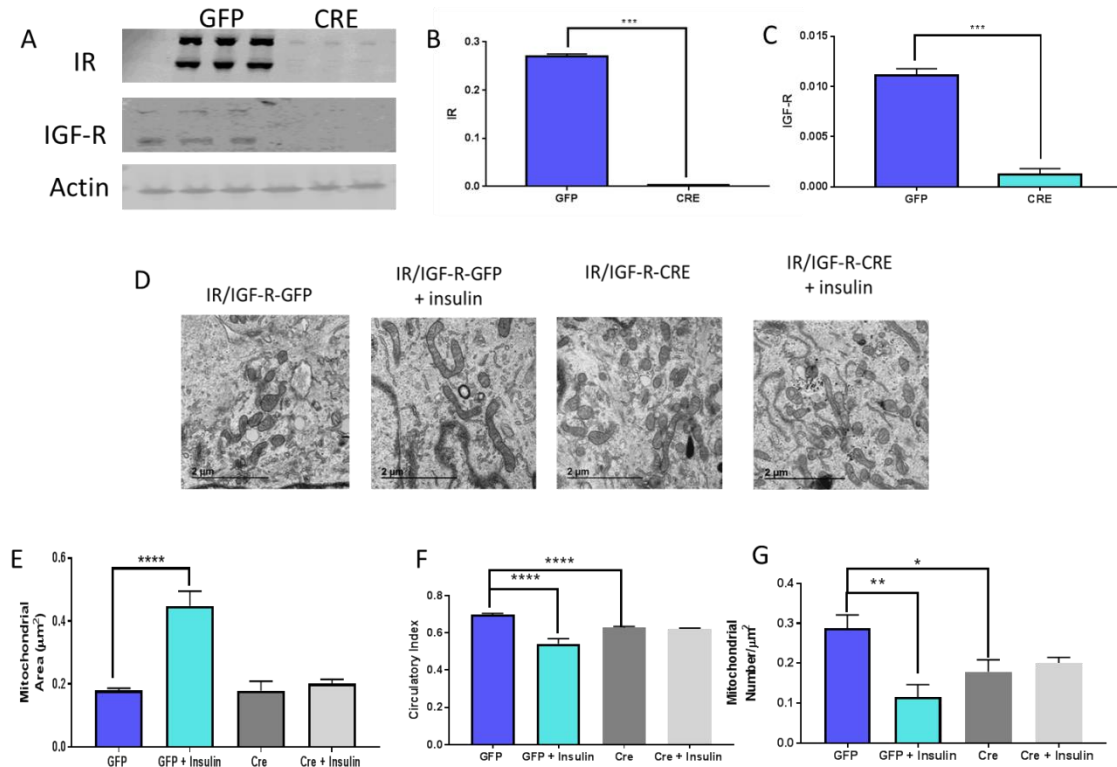


Figure 10. 10A. Western blot of IR and IGF-R after deletion in primary myoblasts. 10B. Western blot Densitometry of IR from Figure 10A after deletion in primary myoblasts. 10C. Western blot Densitometry of IGF-R after deletion from Figure 10A in primary myoblasts. 10D. Representative TEM image of primary myoblasts that received deletion of IGF-R/ IR with or without insulin. Figure 10B- Figure 10D. Circulatory Index (10B), Mitochondrial Area (10C), and Mitochondrial Number (10D) that received deletion of IGF-R/ IR with or without insulin.

Insulin Increases Mitochondrial Fusion in Cultured Primary Myoblasts through an IR-pAKT pathway:

To examine detailed changes in mitochondrial morphology in response to insulin stimulation, we performed electron microscopy analysis in primary myoblasts. The data demonstrated that insulin stimulation for 2 hours decreased circulatory index (Figure 8A and Figure 8B), increased mitochondrial area (Figure 8A and Figure 8C), and decreased mitochondrial number (Figure 8A and Figure 8D). To determine whether the mitochondrial morphology changes are directly related to the effects of insulin on mitochondrial function, we ablated OPA-1 using adenovirus. Interestingly, knockdown of OPA-1 increased circulatory index compared to its control (Figure 8A and Figure B), increased mitochondrial area (Figure 8A and Figure 8C), and did not increase mitochondrial number (Figure 8A and Figure D). Notably, OPA-1 ablation in conjunction with insulin stimulation for 2 hours did not prevent mitochondrial morphology changes. We observed that after 2 hours of insulin stimulation, circulatory index decreased (Figure 8A and Figure 8B), mitochondrial area increased (Figure 8A and Figure 8C), and the number of mitochondria decreased in primary myoblasts (Figure 8A and Figure 8D). This would suggest that insulin might be able to regulate inner membrane fusion independently of OPA-1 ability to mediate mitochondrial metabolism changes. More importantly, to confirm that insulin signaling is required to promote fusion of mitochondria, we ablated IR and IGF-R from primary myoblasts and demonstrated that we were able to successfully ablate IR and IGF-R (Figure 9A – Figure 9C). Next, we performed electron microscopy analysis in ablated IR and IGF-R primary myoblasts that either received insulin stimulation for 2 hours or did not receive insulin stimulation (Figure 9D). The data demonstrated that insulin stimulation for 2 hours decreased circulatory index (Figure 9D and Figure 9E), increased mitochondrial area (Figure 9D and Figure 9F), and decreased mitochondrial number (Figure 9D and Figure 9G). To determine whether the mitochondrial morphology changes are directly related to the effects of insulin on mitochondrial function, we ablated IR and IGF-R using adenovirus and stimulated cells with or without insulin. Notably, after ablation of IR and IGF-R in primary myoblasts, insulin was not able to alter mitochondrial morphology under the following parameters: circulatory index (9E), mitochondrial area (9F), mitochondrial number (9G), (Figure 9E to Figure 9G).

Ablation of OPA-1 Increases ER-Mito Contacts in Myoblasts: It has been shown that OPA-1 deficiency promotes secretion of FGF-21 from muscle that prevents obesity and insulin resistance in a mutant model [2]. Similarly, in skeletal muscle cells, ablation of OPA-1 results in ER stress and FGF-21 protein level increase [2]. Ablation of OPA-1 leads to an increase in the levels of MFN-1 and MFN-2 [2], which are components of the mitochondrial-associated membrane proteins (MAMs). MAMs are proteinaceous tethers that connect the endoplasmic reticulum with mitochondria [3]. Notably, MAMs use a specialized subcompartment at the connects of ER and mitochondria to

maintain efficient transfer of calcium from the ER to Mitochondria, help drive proper mitochondrial bioenergetics, and lipid synthesis [3]. MAMS are believed to regulate mitochondrial shape and motility, and assist with the formation of autophagosome assembly. Most importantly, MAMs have been shown to be a place of significant activity for the transfer of stress signals from the ER to the mitochondria. Interestingly, in our OPA-1 deficient primary myoblasts (Figure 9A and Figure 9B) and OPA-1 deficient primary myotubes (Figure 10 A and Figure 10B), we observed an increase in FGF-21 protein levels (Figure 9C and Figure 9D) and (Figure 10 C and Figure 10 D) and BIP (GRP78), a molecular chaperone (Figure 9 E and Figure 9 F) and (Figure 10 E and Figure 10F). Additionally, ablation of OPA-1 increased expression of MFN-1 and MFN-2 in primary myoblasts (Figure 9G- 9I) and primary myotubes (Figure 10 G- Figure 10 I), a tethering protein. Additionally, we found that loss of OPA-1 increased MCU protein levels and SERCA protein levels in myoblasts (Figure 9J-Figure 9L) in primary myotubes (Figure 10J- Figure 10L). To determine if the loss of OPA-1 increased MAMs, we used electron microscopy. We found that loss of OPA-1 increases the formation of ER-Mitocontacts in primary myoblasts in size of the contact with mitochondria (Figure 11A and Figure 11B), but did not change the size of the interaction with the endoplasmic reticulum (Figure 11A and Figure 11C). Interestingly, the size of each MAM was increased with the deletion of OPA-1 (Figure 11D).

VI. Discussion

Data is going toward publication so we would like refrain from discussing findings.

VII. Conflict of Interest:

Data that was not specifically included will be used toward a scientific manuscript for publication.

VIII. Grant Support

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