# Assessing the Impact of Autophagy on Cellular Metabolism

## Real-Time, Functional Metabolic Assays Connect Energy Pathways and Autophagy

## Application Brief

### Introduction

Autophagy is a highly conserved, homeostatic degradation pathway that involves the breaking down and salvaging of cellular components. Autophagy is a pro-survival process that involves the digestion and recycling of damaged or exhausted organelles, or other cellular components via lysosomes. First described in the 1950s using ultrastructural studies, autophagic mechanism research gained increased interest during the 1990s with the identification of autophagy-related genes (ATG).

Autophagy plays a significant role in cell differentiation, tissue remodeling, and cell proliferation. However, unlike other cell death pathways, such as apoptosis and necrosis, autophagy does not directly result in cell death. Further research into the mechanisms involved in regulating cellular energy investments to maintain cellular structure and function is crucial to prevent unwarranted cell loss. This Application Brief describes the use of Agilent Seahorse XF technology to examine the effects of autophagy on mitochondrial respiration and glycolysis in three recent publications.

Ding; *et al.* (2015)[1] studied mitofusin 2 (Mfn2), a mitochondrial outer membrane protein involved in the regulation of various biological reactions. Mfn2 defects have been observed in obesity, diabetes, and neurodegenerative diseases. Furthermore, Mfn2 deficiency results in impaired autophagic degradation. However, the connection between cell proliferation, autophagy, Mfn2, and metabolism has not been fully elucidated. The authors used Agilent Seahorse XF technology to determine the effects of Mfn2 deficiency and consequently impaired autophagic degradation on the cellular metabolism of HeLa cells.

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Figure 1 shows that the analyses of the Agilent Seahorse XF Cell Mito Stress Test and Agilent Seahorse XF Glycolysis Stress Test illustrate the effects of Mfn2 deficiency, as well as the effect of Rab7 knockdown, which mimics autophagic inhibition on HeLa cell metabolism. The authors observed that while Mfn2 deficiency resulted in a decrease in ATP-linked respiration and an increase in non-mitochondrial respiration, the remaining parameters did not exhibit any effect due to Mfn2 deficiency (Figure 1A). Similar observations were made during the analysis of Rab7 knockdown cells (Figure 1B). The metabolic profile from the Agilent Seahorse XF Glycolysis Stress Test of Rab7 knockdown cells (Figure 1C) revealed a decrease in the overall extracellular acidification rate, a measure of glycolytic activity. Collectively, these data indicate that Mfn2 modulates cellular metabolism, in part, through autophagic degradation.

The Agilent Seahorse XF Cell Mito Stress Test and Agilent Seahorse XF Glycolysis Stress Test were used with an Agilent Seahorse XF24 Analyzer to demonstrate the effects of Mfn2 on mitochondrial respiration and glycolysis. Their analyses determined that Mfn2 deficiency-mediated autophagic impairment disrupts metabolism, and subsequently affects cell proliferation. This study underscores the connection between autophagy and metabolism, and suggests a mechanism that may be applied to Mfn2 deficiency-related diseases.

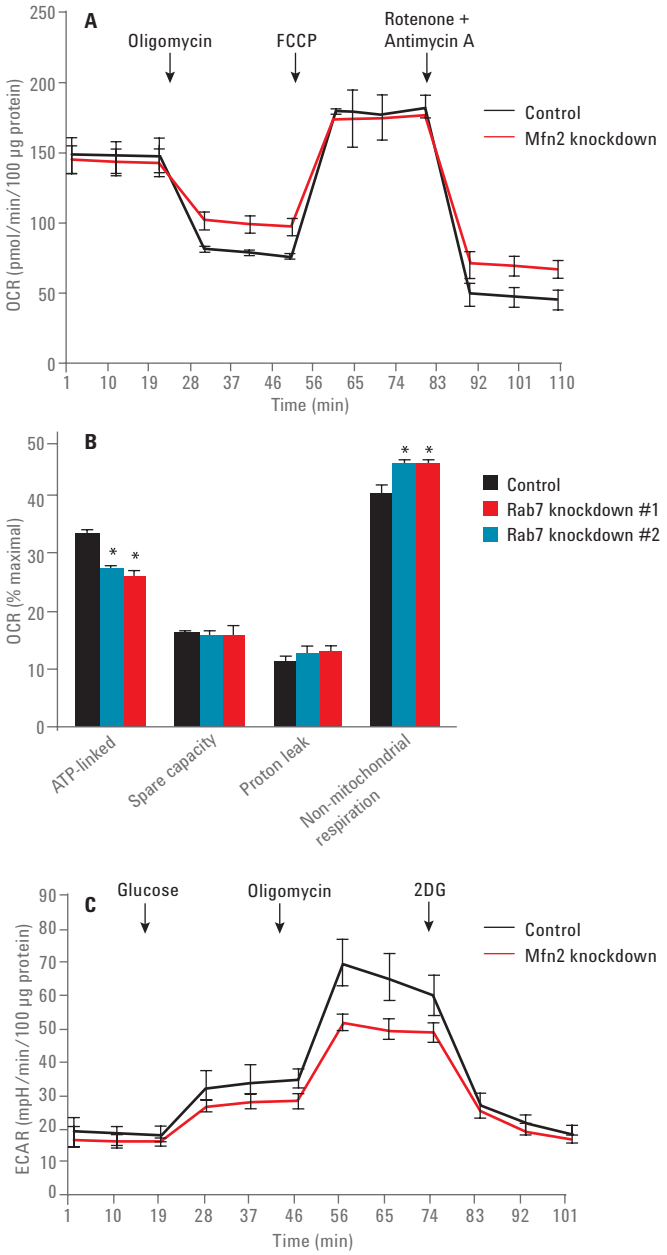


Figure 1. Oxygen consumption and extracellular acidification rates by Mfn2 knockdown or inhibition of autophagic degradation. A and B) Mitochondrial respiration was assessed in Mfn2 knockdown HeLa cells following sequential injections of oligomycin, FCCP, and a combination of rotenone and antimycin A. B) Analysis of mitochondrial respiration parameters. C) Glycolytic activity was assessed following sequential injections of glucose, oligomycin, and 2-DG.

## Results and Discussion

Cells use autophagy to cope with a myriad of stresses to cellular health. Changes to the autophagic pathway have been implicated in the pathology of several diseases, including Alzheimer's disease, aging, autoimmunity, and metabolic syndromes (Mizumura, 2014)[2]. Recent advances in understanding the autophagic processes illustrate the ever-expanding breadth of this field.

Autophagy has been shown to exert protection by removing damaged organelles or cellular components. This protective mechanism provides several beneficial effects including limiting stem cell damage and loss, reducing oncogenic transformation, and reducing age-related immune system decline. Therefore, controlling or modulating autophagic activity is crucial to employing this pathway as a therapy. Stranks; *et al.* (2015)[3] explored the link between autophagy and aged-induced macrophages in an effort to preserve macrophage activity and improve immunological response.

The authors also used an Agilent Seahorse XF24 Analyzer to study macrophages derived from a mouse model. They mimicked aging by deleting an essential autophagy gene, ATG7, and compared the glycolytic activity from the ATG7 knockout and control macrophages. The authors observed a significantly higher basal extracellular acidification rate (ECAR) in knockout macrophages over the control. Based on this data, the authors concluded that the increased rate of glycolysis in the ATG7 knockout macrophages suggests an increased requirement for ATP to either potentially avoid apoptosis, or to compensate for ATP that is normally produced through autophagy. This research highlights the necessity of autophagy in maintaining a functioning immune system.

Lipophagy, a type of selective autophagy, provides free fatty acids and sugars to the cell. Defective lipophagy has been implicated in obesity, atherosclerosis, and non-alcoholic fatty liver disease. Although the role of lipophagy within the cell during nutrient deprivation has not been extensively studied, a recent publication from Rambold; *et al.* (2015)[4] did examine the pathways used by the cell to regulate fatty acid flux during starvation. Using an Agilent Seahorse XF96 Analyzer, the authors first starved mouse embryonic fibroblast cells (MEFs), then inhibited either lipases (enzymes used in the lipolysis of fatty acids), or blocked autophagy. They observed that following a 6-hour starvation, the cells exhibited decreased mitochondrial respiration, regardless of whether either the lipase or the autophagy pathway was inhibited, and after prolonged starvation the cells exhibited a significantly reduced oxygen consumption rate (OCR). These data suggest that acutely stressed cells derive fatty acid substrates primarily from lipolysis, the process of breaking down lipids. The authors concluded that discerning the mechanisms regulating fatty acid flux would substantially contribute to a greater understanding of the pathophysiology of lipid-associated diseases, including obesity, diabetes, cancer, and inflammatory disorders.

## Materials and Methods

HeLa cells were cultured with DMEM supplemented with 10 % Fetal Bovine Serum (FBS). To generate the Mfn2 knockout cell line, HeLa cells were infected with an adenovirus construct containing either the control scramble or shRNA for Mfn2. Additionally, to generate Rab7 knockout cell line, HeLa cells were transfected with either control scramble or siRNA.

### XF Analysis

Metabolic analyses were performed using the Agilent Seahorse XF24 Analyzer, which enables the real time, simultaneous rate measurements of OCR and ECAR by creating a transient microchamber within each well of specialized cell culture microplates. Figure 2 shows HeLa cells were seeded in Agilent Seahorse XF24 Cell Culture Microplates.

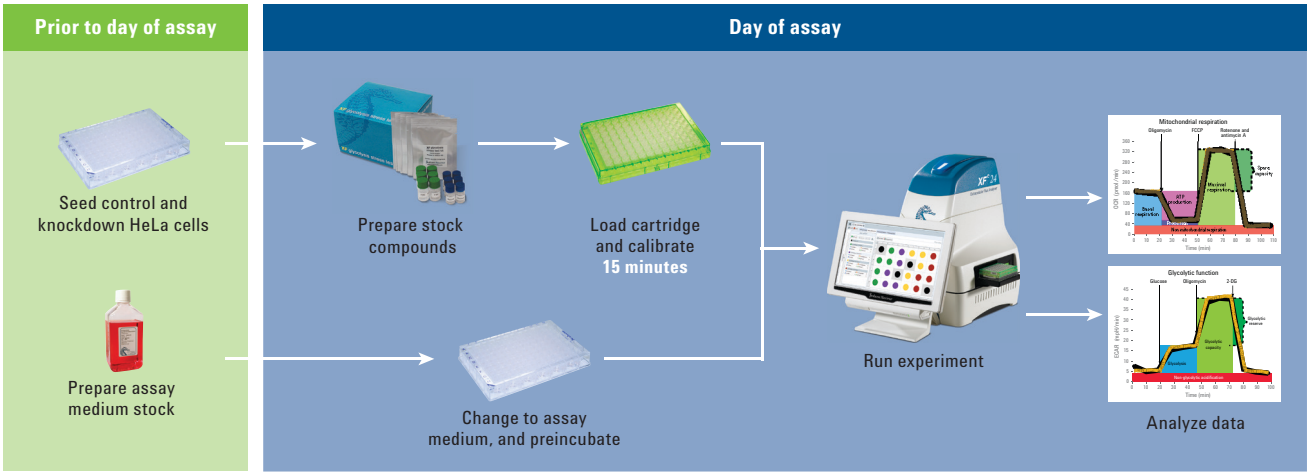


Figure 2. Flow chart of the XF assay.

For the Agilent Seahorse XF Cell Mito Stress Test, cell culture medium was exchanged for Agilent Seahorse XF Base Medium supplemented with 25 mM glucose and 2 mM pyruvate. Following instrument calibration, basal OCR was measured followed by sequential injections of oligomycin, FCCP, and a combination of rotenone and antimycin A. For the Agilent Seahorse XF Glycolysis Stress Test, culture medium was exchanged for Agilent Seahorse XF Base Medium supplemented with 2 mM L-glutamine. Following basal ECAR measurement, cells were sequentially injected with glucose, oligomcyin, and 2-deoxy-D-glucose (2-DG). Each experiment had at least three biological replicates, and both OCR and ECAR values were normalized to 100 µg of protein in each well.

## References


1. Ding; *et al.*, Mitofusin 2-deficiency suppresses cell proliferation through disturbance of autophagy. *PLoS One* **2015**, *10(3)*, e0121328.

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