# Oxygen Consumption Rates Between Skeletal Muscle Cells Derived from Young and Old Human Donors Elucidate Mitochondrial Dysfunction

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## ABSTRACT

Mitochondrial oxidative phosphorylation plays a significant role in cellular functions such as nutrient metabolism, ATP synthesis, and respiratory capacity. Mitochondria's branching network, the reticulum, is active through the fusion and fission dynamics. As humans age, however, there is a loss in the ability of the mitochondria to maintain its structural integrity. Therefore, the purpose of this study is to investigate mitochondrial dysfunction in human skeletal muscle cells (SkM) derived from young and old human donors. In order to determine mitochondrial dysfunction, oxygen consumption rate (OCR) was measured using the Cell Mito Stress Test by SeaHorse Analytics XFp Analyzer (Agilent Technologies; Santa Clara, CA) between SkM from 18M and 66M. Immortalized C2C12 mouse skeletal muscle cells were used as a control to compare the primary SkM with a cell line. Basal OCR and Maximal OCR were higher in 18M compared to 66M (Basal: 28.51 ± 1.61 and 20.43 ± 2.18; Maximal: 54.98 ± 6.74 and 28.68 ± 3.91 pmol/min). Additionally, ATP Production and Spare Respiratory Capacity (SRC) were higher in 18M compared to 66M (ATP Production: 23.88 ± 1.37 and 16.84 ± 2.04 ; SRC: 26.47 ± 5.13 and 8.25 ± 1.73 pmol/min). The discrepancies in the OCR between the young and the old SkM augment our understanding of how mitochondrial dysfunction may serve as a driving force behind aging in humans. Thus, this study will elucidate the various mechanisms that propel age-related mitochondrial dysfunction and provide crucial information to prevent skeletal muscle pathologies.

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## INTRODUCTION

Mitochondria are at the center of cellular metabolism regulation. Thus, it is paramount to acknowledge the important role mitochondria play in biological aging. Mitochondrial dysfunction contributes significantly to age-related conditions and diseases; therefore, disruption of mitochondrial homeostasis can serve as a hallmark of aging. Some characteristics of biological aging include oxidative damage or intracellular debris which can progressively damage mitochondria.[1] For instance, oxidative stress can lead to progressive accumulation of reactive oxygen species (ROS) which results in cellular damage that can affect downstream cellular processes. Electrons are passed through the electron transport chain through electronegative electron-acceptors (oxidizing agents) to establish a proton gradient across the inner mitochondrial membrane; molecular oxygen is critical for the efficient generation of ATP and also serves as the primary source of ROS.[2] Aging mitochondria display morphological abnormalities and impaired ATP synthesis concomitantly with increased ROS-induced damage. Additionally, mtDNA compared to nuclear DNA is more vulnerable to oxidative damage caused by ROS due to the close proximity to the site of production. Because of this, there is approximately a 20-fold increase in mutation rates from deletions or duplications that accumulate in aged human skeletal muscle cells.[3] Another reason why ROS production is detrimental to skeletal muscle is due to its sensitivity against it. Synthesis as well as degradation and satellite cell function- which are major growth regulators- are dependent on reliable homeostatic mitochondrial function.[4] Thus, overproduction of ROS with age can subsequently lead to oxidative damage, apoptosis, and loss of mitochondrial membrane potential which contributes tovarious age-related pathologies, including sarcopenia, Parkinson's Disease, and other neurodegenerative conditions.[5]

Another contributing factor of ROS is the failure to adequately clear biological waste. There are various proteins such as 26S proteasome and PERK-mediated UPR (protein kinase R like endoplasmic reticulum kinase mediated unfolded protein response) that are involved in mitochondrial proteostasis.[6] Nevertheless, lack of cellular clearance can lead to misfolded proteins and aggregation of waste, which can lead to cell death; ablating genes that encode proteins regulating waste transportation to lysosomes, also called autophagy, is correlated with mitochondrial dysfunction.[7] Overaccumulation of ROS perpetuates homeostatic imbalance, which weakens lysosomal degradative capacity as well as recycling of damaged mitochondria.8

## STUDY OBJECTIVE

The purpose of this study is to investigate mitochondrial dysfunction in human skeletal muscle derived cells (SkM) derived from young and old human donors.

## METHODS

### *Skeletal Muscle Myoblasts and Culture*

Skeletal muscle derived cells (SkM) were obtained from Cook Myosite (Pittsburgh, PA), which maintains primary skeletal muscle cells from donors from various age ranges. For the young sample group, SkM derived from healthy 18 and 19-year-old donors (18M, 19M) were used. For the old sample group, SkM derived



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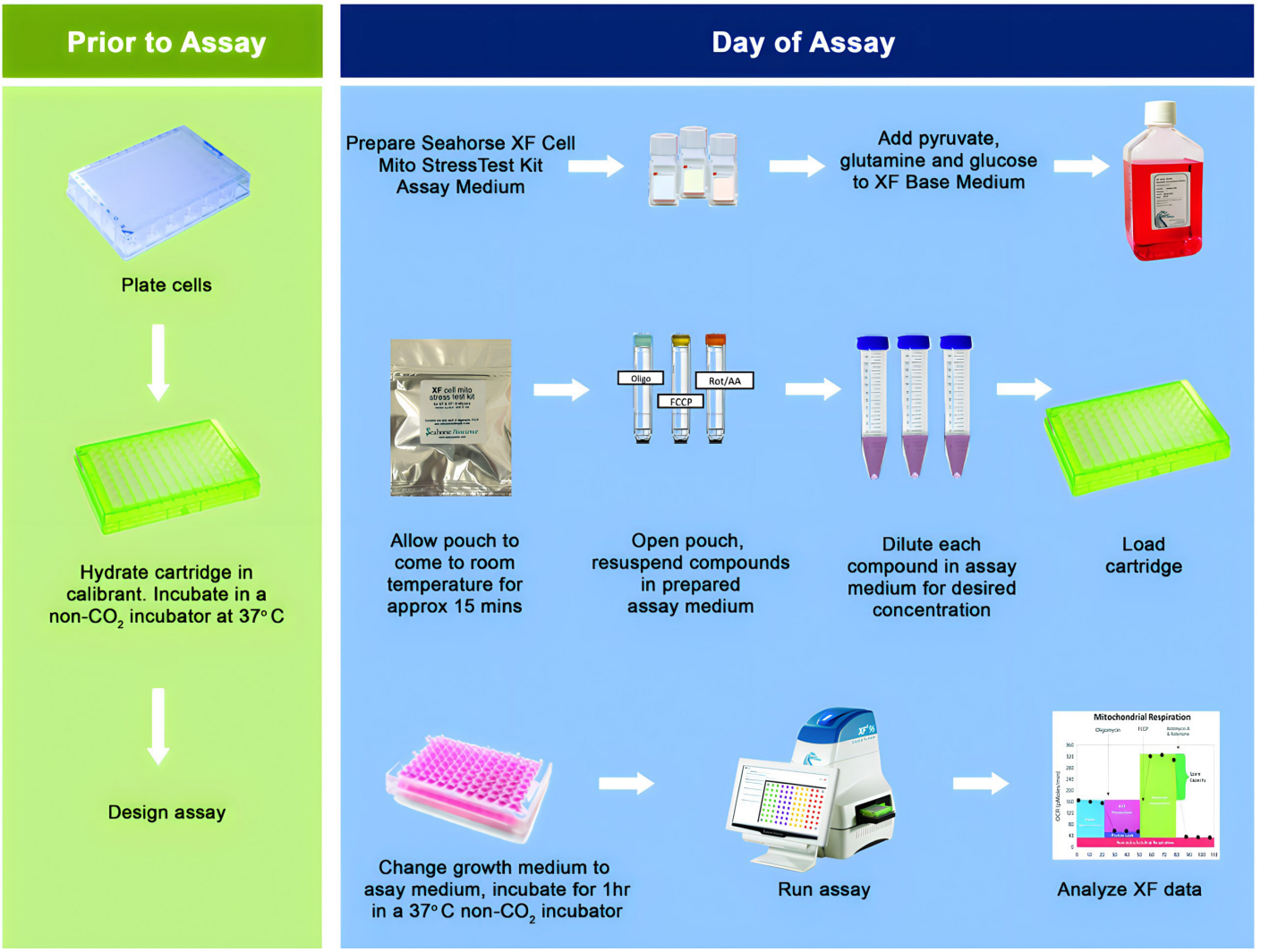


***Figure 1: Agilent Seahorse XF Cell Mito Stress Test Assay Workflow.*** *Taken from Agilent Seahorse Analytics.*

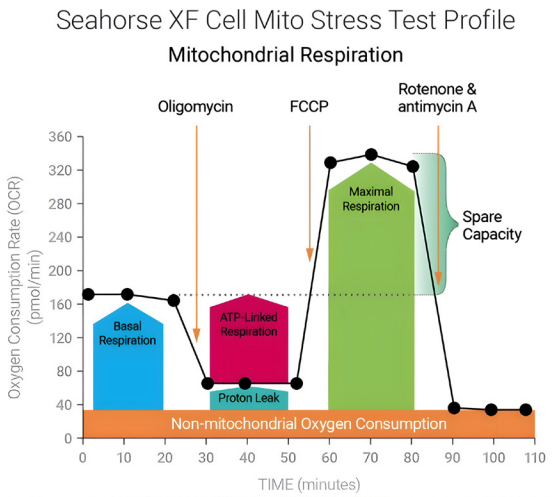


***Figure 2: Seahorse XF Cell Mito Stress Test Profile - reagents Oligomycin, FCCP, Rotenone and Antimycin A used to measure OCR under various conditions.*** *Taken from Agilent Seahorse Analytics.*

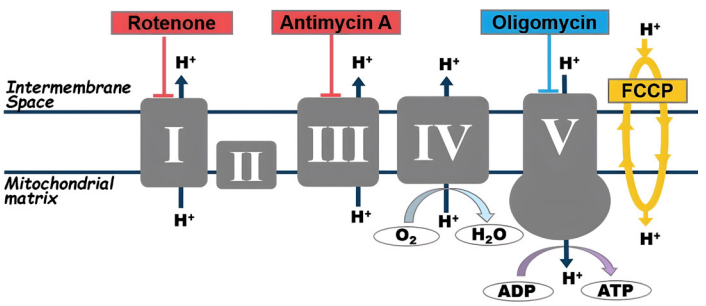


***Figure 3: Different reagents block the electron transport at different stages; the uncoupling reagent allows for proton influx into the mitochondrial matrix.*** *Taken from Agilent Seahorse Analytics.*

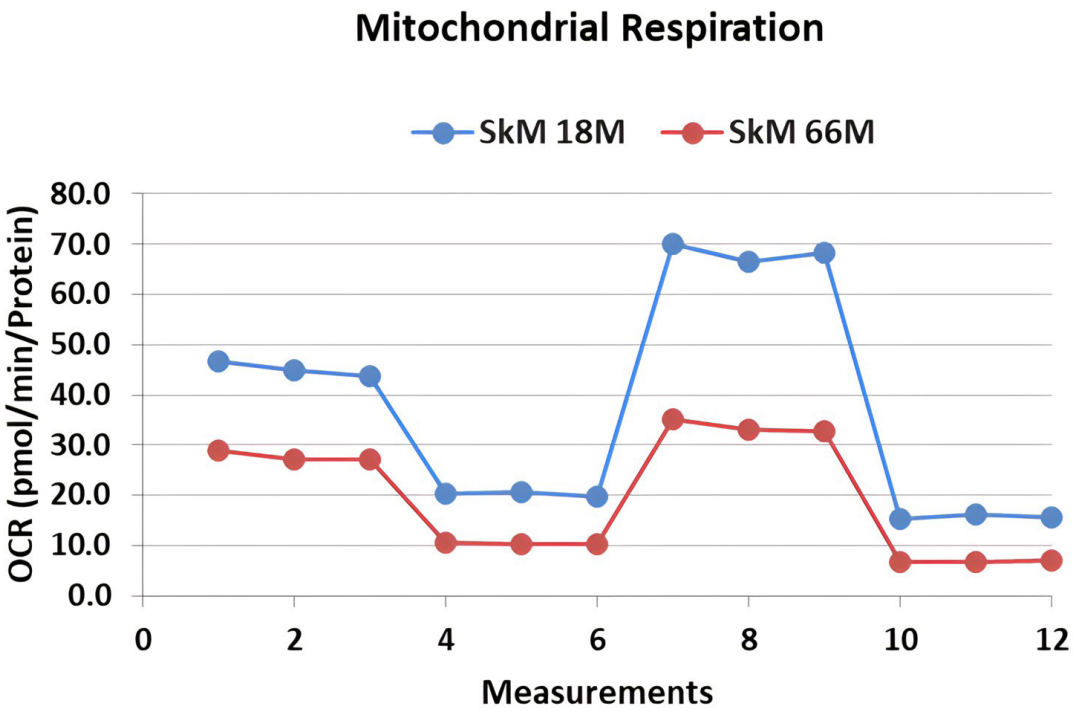


*Figure 4: OCR of the 18M versus 66M SkM.*

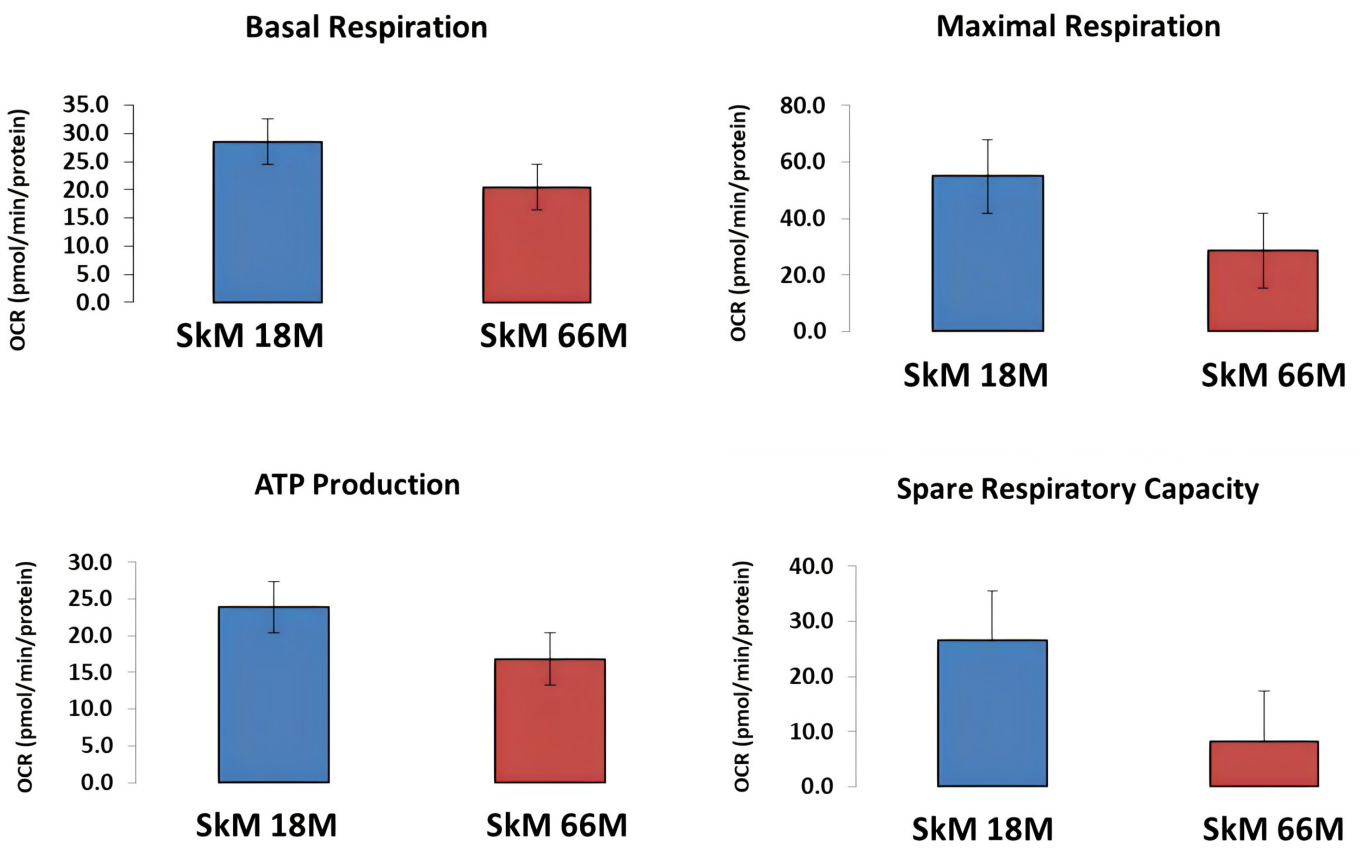


*Figure 5: Comparisons of the Basal Respiration, Maximal Respiration, ATP Production, and Spare Respiratory Capacity of the SkM 18M versus 66M.*

from a healthy 66-year-old donor (66M) were used. As a control, immortalized mouse C2C12 myoblasts were used to compare the data from the primary SkM to that of a cell line.

Cells were maintained in <70% confluency and low passage numbers and were given Ham's F10 Nutrient Mix containing 20% Fetal Bovine Serum (FBS), 1% Antibiotic/Antimycotic (A/A), and 10mM HEPES buffer.

***Mitochondrial Respiration Assay***

Oxygen Consumption Rate (OCR) serves as a proxy for any underlying mitochondrial dysfunction. The Seahorse Cell Mito Stress Test (Agilent Technologies; Santa Clara, CA) was run on the XFp Analyzer to measure the discrepancies in OCR of mitochondria in the adhered cells. This machine measures key parameters of mitochondrial respiration in real time on a small number of cells with no radioactivity.[9] Figure 1 contains the workflow for the Cell Mito Stress Test.

Before running the assay, the cell culture medium was exchanged with the Seahorse assay medium. Various injections of drugs were given in order to specifically target certain metabolic processes. The advantage of using the Seahorse Analytics is that various parameters can be measured in one assay: basal respiration, maximal respiration, ATP production, and reserve capacities. The Cell Mito Stress Test Kit provides oligomycin, carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP), as well as rotenone/antimycin A (Figure 6). After measuring the basal OCR, the injection of oligomycin inhibits ATPase. As a result, due to lack of ATP production, OCR decreases. FCCP serves as an uncoupling agent of oxidative phosphorylation and disrupts the mitochondrial membrane potential allowing protons to enter the inner membrane. Thus, FCCP facilitates maximal respiration by increasing H+ transport across the inner membrane. As a result, oxygen consumption occurs without being coupled to ATP production. Rotenone/antimycin A inhibits complexes I (rotenone) and III (antimycin A) of the mitochondrial respiratory chain to entirely halt mitochondrial electron transfer and respiration altogether (Figure 2 and 3).

## RESULTS

***Cell Mito Stress Tests***

The Seahorse XF instrument measures OCR in real time at intervals of approximately 5-8 minutes post-injection of the reagents. The Seahorse Analytics Report Generator (Mean±SEM) revealed that Basal OCR and Maximal OCR were higher in 18M compared to 66M (Basal: 28.51 ± 1.61 and 20.43 ± 2.18; Maximal: 54.98 ± 6.74 and 28.68 ± 3.91 pmol/min). In addition, ATP production and Spare Respiratory Capacity (SRC) were higher in 18M compared to 66M (ATP Production: 23.88 ± 1.37 and 16.84 ± 2.04 ; SRC: 26.47 ± 5.13 and 8.25 ± 1.73 pmol/min). Refer to Figures 4 and 5.

Basal OCR and maximal OCR were higher in C2C12 cells compared to 18M (Basal: 28.40 ± 1.91 vs.9.99 ± 0.24; Maximal: 77.83 ± 1.34 vs. 15.24 ± 0.31pmol/min). ATP production was also higher in C2C12 compared to 18M (C2C12: 13.42 pmol/min vs. 18M: 5.42 pmol/min). See Figures 6 and 7.

## DISCUSSION

The Cell Mito Stress Test revealed that Basal OCR, Maximal OCR, ATP Production, and SRC of the 18M cells were higher than those of the 66M cells. The discrepancies in the OCR between the young versus the old human cells, therefore, add to our understanding of how mitochondrial dysfunction may serve as a driving force behind aging in humans. In addition, the C2C12 immortalized myoblasts demonstrate a higher OCR compared to primary human skeletal muscle derived cells; this can be accounted for by the fact that the C2C12 cells are programmed to continually divide, which require more energy production compared to the human cells. Mitochondria are crucial players in the aging process, and a plethora of aging-related pathophysiologies have mitochondrial dysregulation components. The architecture of mitochondria, which are primarily driven by active fusion and fission, serves as a critical axis of mitochondrial quality control and sustenance of metabolic functions in cells. It is critical to acknowledge the versatile role of mitochondria in the maintenance of human health. Perturbations to the mitochondria can significantly impact mitochondrial dynamics, ROS production, as well as energy and nutrient metabolism. Consequently, altered homeostatic balance of mitochondrial morphology and dynamics contributes to a myriad of age-related conditions. Thus, this project reveals the importance of regulation and coordination of mitochondrial dynamics for cellular homeostasis; the results from the study elucidates how discrepancies in the OCR of mitochondria between the young versus old human cells reflect mitochondrial dysfunction that occurs with biological aging.

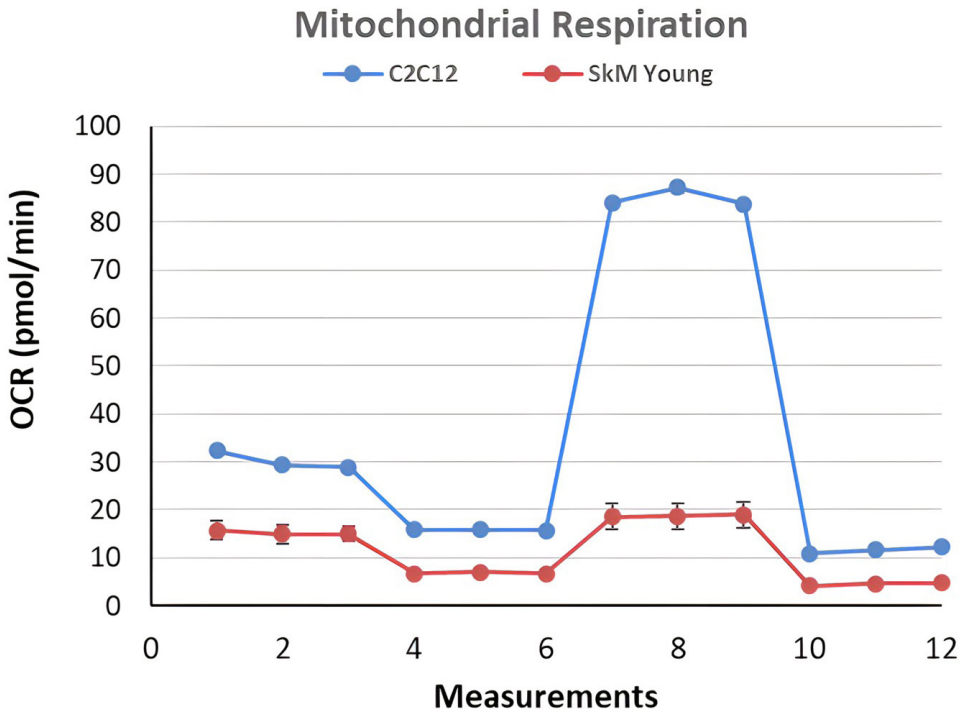


*Figure 6: OCR between the C2C12 cells versus SkM 19M.*

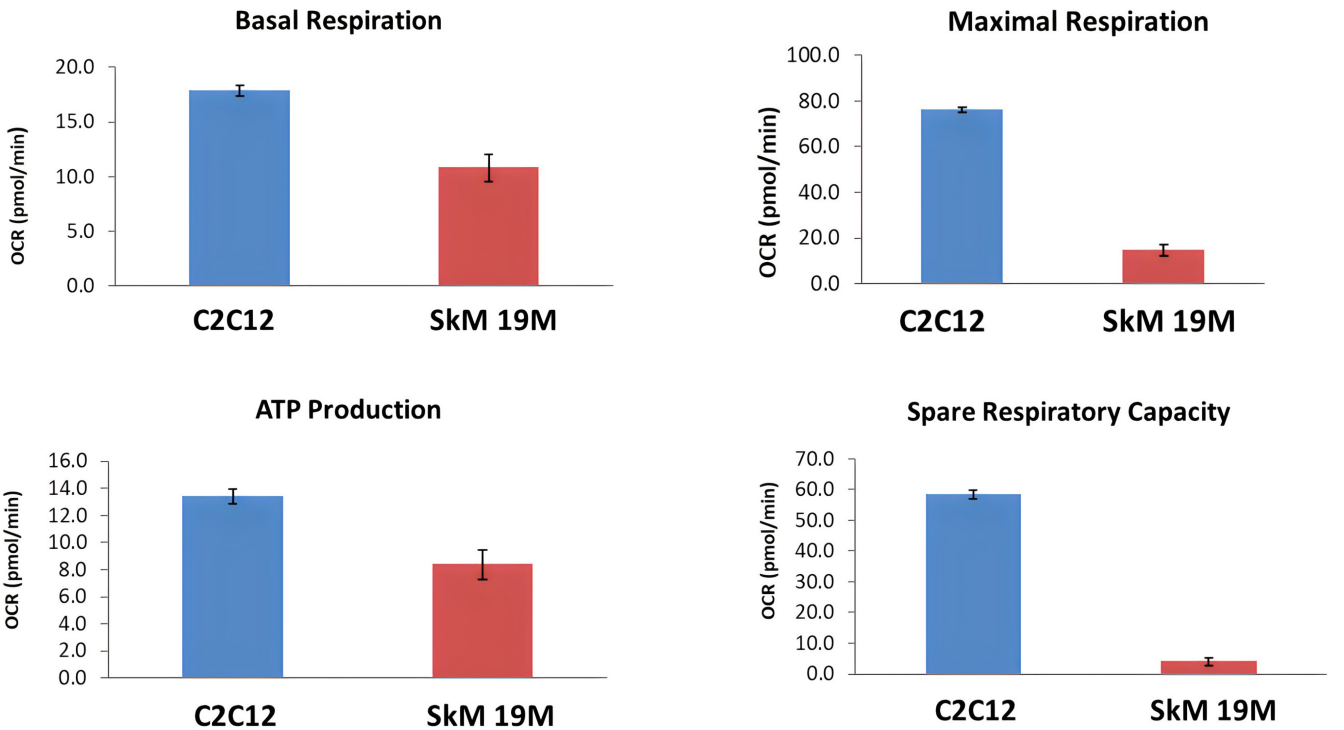


*Figure 7: Comparisons of the Basal Respiration, Maximal Respiration, ATP Production, and Spare Respiratory Capacity for the C2C12 immortalized cells versus SkM 19M.*

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