Oxygen Consumption Assay

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Tradename: ACB Yogurt Dermal Respiratory Factor PF

Code: 20224PF

CAS #: 68333-15-3

Test Request Form #: 14451

Lot #: 8778300

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Test Performed:
Oxygen Consumption Assay

Introduction

Cellular respiration occurs within the mitochondria and is defined as a series of metabolic processes where oxygen (O_2) is consumed/utilized to harvest biochemical energy from organic substances that is stored in energy-carrying biomolecules (adenosine triphosphate (ATP)). ATP is vital to cellular function as it fuels molecular transport across membranes, cellular migration and proliferation, biosynthesis of necessary cellular components, and provides energy for other biochemical reactions. Efficient O_2 consumption improves energy production and reduces the amount of harmful reactive oxygen species (ROS) that can cause damage to the cell and surrounding tissues. Excessive and unregulated oxidative stress leads to aging and impaired cellular function which manifests as wrinkles, loss in elasticity, unwanted pigmentation, and inflammation. Assisting in the efficiency of O_2 consumption also aids in attenuating ROS being produced within the system which will ultimately reduce premature cellular aging.

An Oxygen Consumption Assay was conducted to assess the *in vitro* effect of **ACB Yogurt Dermal Respiratory Factor PF** to enhance oxygen consumption in isolated mitochondria from human dermal fibroblasts. Augmenting cellular respiration maintains skin homeostasis and counteracts the age-related decline in skin cell function.

Assay Principle

Mitochondria are isolated from human dermal fibroblasts and placed in a heated respiration chamber where oxygen consumption is monitored with a Clark electrode. Succinate, a metabolic intermediate for Complex II of the election transport chain, is added to the respiration chamber to initiate cellular respiration which provides a basal level of oxygen consumption. Test articles are added to the respiration chamber and changes in oxygen consumption are assessed compared to basal oxygen consumption.

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Materials

A. Kit:	Mitochondria Isolation Kit (Millipore-Sigma; MITOISO2-1KT)*
B. Incubation Conditions:	37°C, 5% CO_2, and 95% relative humidity (RH)
C. Equipment:	Forma Humidified Incubator; ESCO Biosafety Laminar Flow Hood; Pipettes; Light Microscope; Centrifuge; Dounce Homogenizer; Respiration Chamber; Clark Oxygen Electrode; Magnetic Stir Bar; Hamilton Syringe; Teflon Membrane; Mesh Paper; O-rings
D. Cell Line:	Normal Human Dermal Fibroblasts (ATCC; PCS-201-012)*
E. Media/Buffers:	Fibroblast Basal Medium (PCS-201-030)*; Fibroblast Growth Kit (PCS-201-041)*; Phosphate Buffered Saline (PBS); Assay Buffer (70 mM Sucrose, 220 mM Mannitol, 2 mM HEPES [pH 7.4], 5 mM MgCl, 5 mM Potassium Phosphate, 1 mM EDTA, 0.1% BSA); Sodium Dithionite; 5 mM Succinate; Electrode Buffer
F. Reagents:	Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) (Sigma Aldrich, C2920)*; Antimycin A (Sigma Aldrich, A8674)*
G. Culture Plate:	T75 Tissue Culture Treated Flasks
H. Software:	Oxygraph Software
I. Other:	Sterile disposable pipette tips

*Or suitable alternatives, subject to change without notice based off vendor availability

Methods

Human dermal fibroblasts were seeded into a T75 tissue culture flask and grown to confluency in complete media. The cells were harvested, centrifuged, and resuspended in PBS, followed by another centrifugation and resuspension in extraction buffer. After an incubation on ice, the cells were lysed via homogenization and the isolated mitochondria were pelleted via centrifugation and resuspended in assay buffer.

The respiration chamber was heated to 37°C and equilibrated for 30 minutes prior to experiments. Cathodes were inspected to ensure the absence of tarnish, after which electrode buffer was applied to the cathodes and covered with mesh paper, a Teflon membrane, and secured with an O-ring. The electrode and respiration chamber were combined, and a magnetic stir bar was placed in the bottom of the chamber. PBS was added to the chamber and the magnetic stir bar was set to 30% of maximum speed. The chamber and electrode were equilibrated for 30 minutes. For calibration, the Oxygraph Software monitored temperature and oxygen concentration for 30 minutes. After the oxygen concentration was stable, the electrode was calibrated to indicate air saturated PBS. Next, sodium dithionite was added to the chamber to remove all free oxygen and achieving a stable oxygen reading represented zero oxygen. The chamber was then flushed several times with PBS to remove all sodium dithionite.

The isolated mitochondria and 5 mM succinate were added to the chamber. Oxygen concentrations were monitored for 15 minutes to obtain a basal oxygen consumption level. After basal measurements, **ACB Yogurt Dermal Respiratory Factor PF** was added to the chamber at a final concentration of 2.0% and oxygen consumption was further monitored for five minutes. FCCP and Antimycin A were utilized as positive and negative controls, respectively. FCCP is a mitochondrial uncoupler and elicits maximum oxygen consumption, whereas Antimycin A is a Complex III inhibitor and arrests cellular respiration. Experiments were performed in duplicate. Oxygen consumption rates were obtained from the Oxygraph Software and are displayed as a percent of basal readings calculated by the following equation:

$$Oxygen\ Consumption\ (\%\ of\ Basal) = \frac{Oxygen\ Consumption_{Test\ Article}}{Oxygen\ Consumption_{Basal}} \times 100$$

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Results

The data obtained from this study met criteria for a valid assay and the positive and negative controls performed as anticipated. Compared to baseline oxygen consumption, isolated mitochondria from dermal fibroblasts in the presence of 2.0% **ACB Yogurt Dermal Respiratory Factor PF** demonstrated an increase in oxygen consumption.

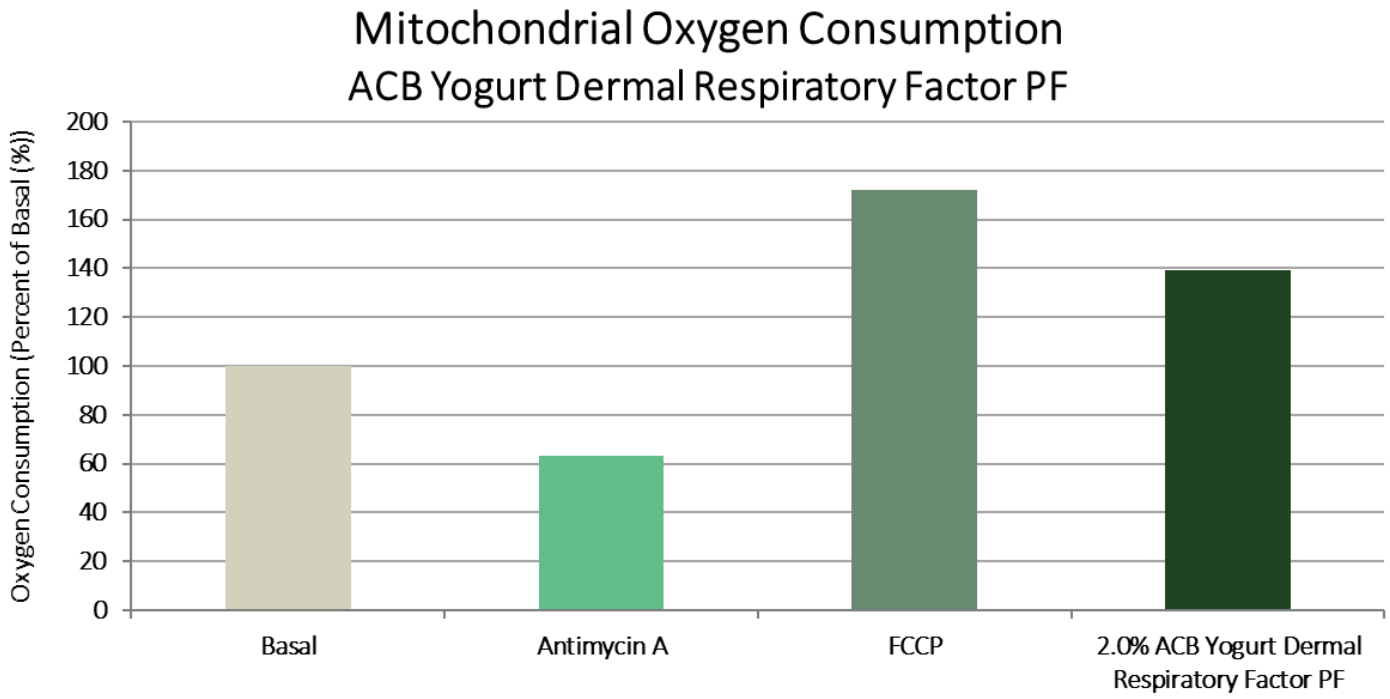

Figure 1. The effect of **ACB Yogurt Dermal Respiratory Factor PF** on oxygen consumption in isolated mitochondria from dermal fibroblasts.

Discussion

As shown in Figure 1, isolated mitochondria from dermal fibroblasts exposed to Antimycin A, a known cellular respiration inhibitor, exhibited a 37% reduction in oxygen consumption compared to basal levels. Conversely, mitochondria subjected to FCCP, a known cellular respiration stimulant, elicited a 72% increase in oxygen consumption compared to basal levels. These data demonstrate cellular respiration in isolated mitochondria from dermal fibroblasts is dynamic and can be manipulated with exogenous compounds.

Similarly, isolated mitochondria from dermal fibroblasts exposed to 2.0% **ACB Yogurt Dermal Respiratory Factor PF** demonstrated a 39% increase in oxygen consumption compared to basal levels. This data demonstrates **ACB Yogurt Dermal Respiratory Factor PF** augments cellular oxygen consumption.

Collectively, oxygen consumption is a biological process that generates the energy required for cellular functions such as cellular migration and proliferation, biochemical reactions, molecular transport across membranes, and biosynthesis of cellular components. Maintaining efficient cellular respiration limits oxidative stress production that can induce damage in surrounding cells and tissues. These data indicate **ACB Yogurt Dermal Respiratory Factor PF** enhances cellular oxygen consumption, which may help to attenuate characteristics of cellular aging by improving cellular respiration efficiency.