# Metabolic Profile in Monocytes from PLWH and Role of cART in Increasing Mitochondrial Respiration and Glycolysis

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## Abstract

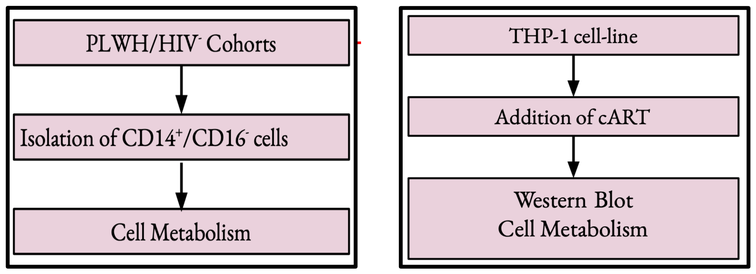
Human Immunodeficiency Virus (HIV) is a retrovirus that attacks the immune cells of the body, specifically CD4⁺ T cells, macrophages, and dendritic cells. To mitigate viral replication in patients, combined antiretroviral therapy (cART) is administered daily. Such treatment, while not completely eliminating the virus from infected individuals, greatly increases the life span of these patients and reduces the risk of further transmission through a reduction of viral load and restoration of the CD4⁺ T cell count. However, People Living with HIV (PLWH) undergoing cART still have a dysfunctional immune system that may lead to secondary illnesses, including cancer.

Monocytes and macrophages are key players of the innate immune system. When monocytes are exposed to consecutive treatments with microbial products such as the fungal β-glucan or the lipopolysaccharides (LPS) of gram-negative bacteria, epigenetic and metabolic remodeling lead to the development of trained or tolerant phenotypes. Trained cells are characterized by an increased production of pro- inflammatory cytokines (called hyper-responsiveness), such as IL-6, IL-1β, and TNF-α. In contrast, tolerant phenotypes are characterized by hypo-responsiveness upon the same exposure to LPS.

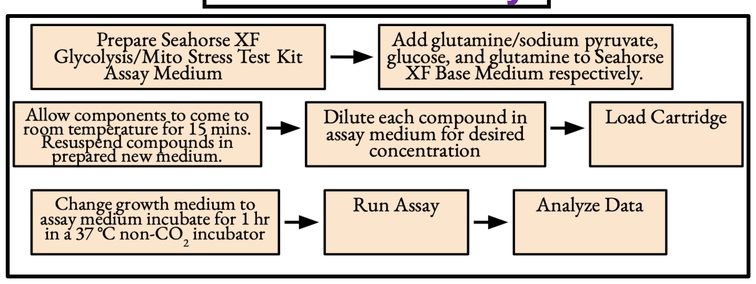
IKAROS, a transcription factor encoded by the IKZF1 gene, is a master regulator of hematopoiesis, as well as lymphocyte differentiation and function. Our laboratory has previously found that monocytes from PLWH display hyper-responsiveness to consecutive LPS treatments and are incapable of establishing a tolerant phenotype compared to HIV⁻ control cells. Furthermore, our laboratory has identified IKAROS as a critical factor in this imbalanced immune response. Since trained immunity is linked to metabolic reprogramming, we sought to investigate whether cART can alter the metabolism of monocytes and whether IKAROS has a role in the cART-mediated effects.

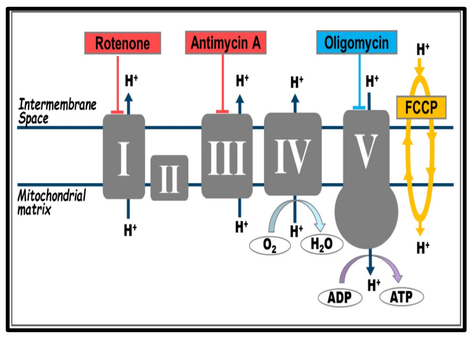
We utilized CD14⁺/CD16⁻ monocytes from PLWH and HIV⁻ controls, as well as the THP-1 monocytic cell line and found that monocytes from PLWH or from HIV⁻ controls treated with cART had increased mitochondrial respiration and glycolysis compared to untreated controls. In addition, THP-1 cells treated with cART displayed a metabolic profile comparable to the one of PLWH. Similar to our previous data obtained in PLWH, Western blot analysis of THP-1 cells treated with cART showed a reduced expression of IKAROS. Altogether, our data suggest that cART could be responsible for the increased metabolism in PLWH. Future experiments are aimed at understanding the role of IKAROS in the cART-induced dysfunctional responses in monocytes.

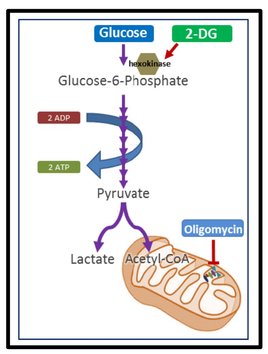
## Materials and Methods

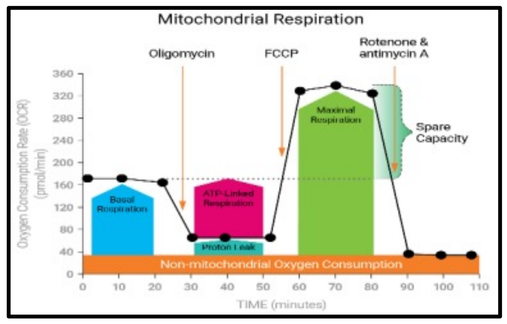


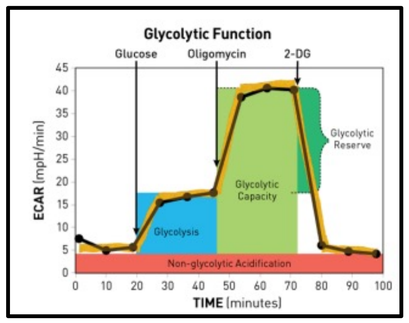

### Seahorse Assay



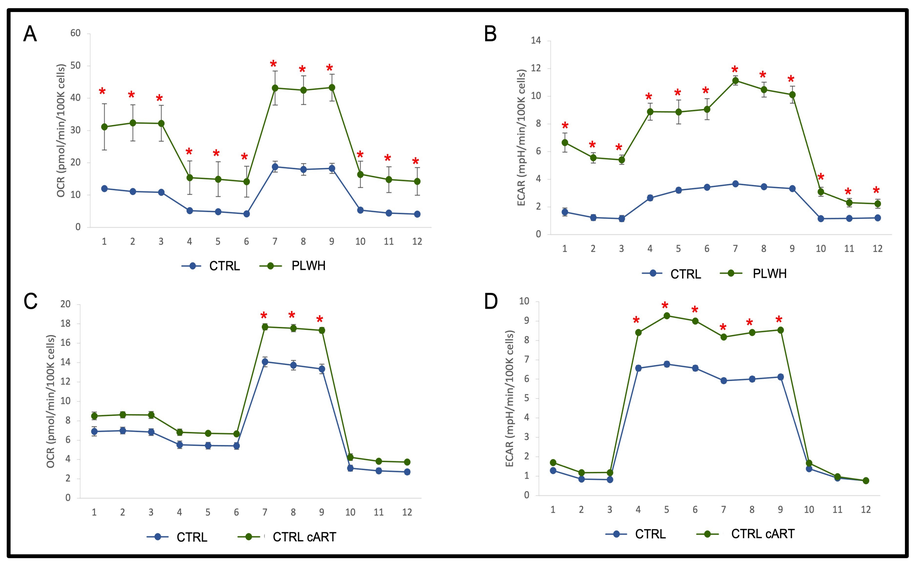



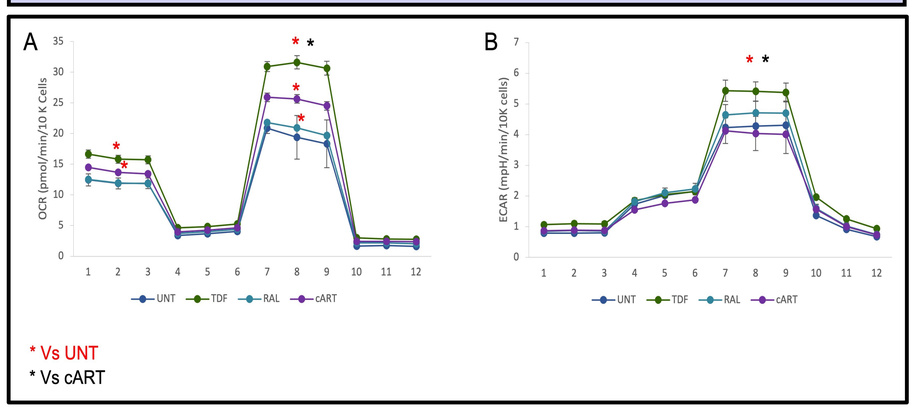

**During the Seahorse assay, three drugs are injected at various time intervals into the cell plate from their respective ports. Each drug is prepared at a predetermined concentration. For the Mito stress test, the following drugs are injected sequentially: Oligomycin, FCCP, and Rotenone/Antimycin A. For the Glycolysis stress test, three different drugs are injected sequentially: glucose, oligomycin, and 2D-G. Injection of these drugs allow for a measure of the respiratory (Oxygen Consumption Rate - OCR) and glycolytic rates (Extracellular Acidification Rate - ECAR), respectively.**

## Increased metabolic activity of PLWH compared to HIV⁻ controls



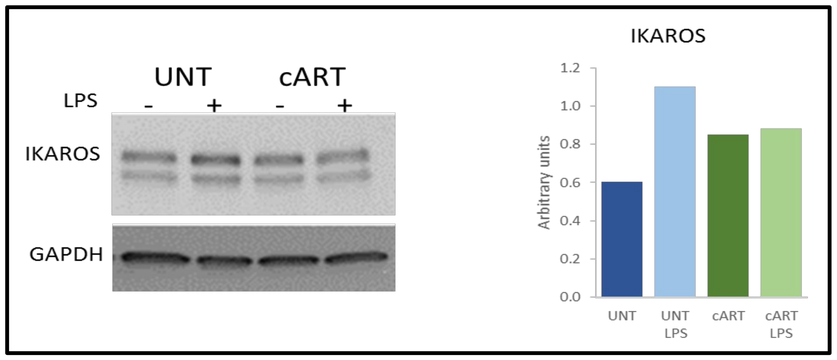

**CD14⁺/CD16⁻ cells were isolated from PLWH and HIV⁻ control and were used to assess the mitochondrial respiration (mitochondrial stress test) and the glycolysis (glycolysis stress test) through Seahorse assays. (A) shows OCR and (B) shows ECAR of PLWH compared to HIV⁻ control. (C, D) CD14⁺/CD16⁻ cells extracted from HIV⁻ control were untreated or treated with cART for 4 days and OCR and ECAR were measured respectively. cART is a combination of Emtricitabin (EMT, 40nM), Tenofovir Dysoproxil Fumarate (TDF, 500nM), and Raltegravir (RAL, 4nM). SEM was calculated from a minimum of 4 replicates. Asterisks indicate statistical significance.**

## THP-1 cells treated with cART show a metabolic profile similar to monocytes from PLWH




**THP-1 cells were used to assess mitochondrial respiration and glycolysis. (A) shows the OCR and (B) shows ECAR profiles of THP-1 cells treated for 3 days with TDF, RAL, or cART (as above) compared to untreated control THP-1 cells. SEM was calculated from a minimum of 4 replicates. Asterisks indicate statistical significance.**

## Effect of cART on IKAROS Expression in THP-1 cells




**To mimic an inflammatory response, THP-1 cells were treated with cART for 3 days and then LPS was added overnight. The following day cells were harvested and proteins were extracted with RIPA buffer. Western blot was used to assess the expression of IKAROS. GAPDH was used as loading control. The graph shows the densitometric analysis of the bands relative to IKAROS after normalization with GAPDH obtained through Image J software. Similar to what we observed in monocytes from PLWH, these results indicate that cART can attenuate the expression of IKAROS during the inflammatory response, with negative consequences on the upregulation of factors involved in the negative regulation of inflammation.**

## Results

- PLWH showed increased metabolic activity as measured by OCR and ECAR.
- A similar increase in respiratory rate and glycolytic rate was seen when control HIV⁻ primary cells and THP-1 cells were treated with cART.
- Treatment of THP-1 cells with TDF resulted in a metabolic profile that mimicked cART.
- To understand how cART affects expression of IKAROS, Western blot assays revealed an increase in IKAROS expression when THP-1 cells were treated with lipopolysaccharide (LPS). However, a similar increase was not seen when cells were treated with cART. As such, cART appears to inhibit the upregulation of IKAROS during an inflammatory response.

## Discussion

Our laboratory has found that monocytes from PLWH have a dysregulation of the immune response because of an imbalance between positive and negative regulators of inflammation due, at least in part, to a reduced expression of IKAROS [Faia et al, 2021]. As such, monocytes from PLWH demonstrate a trained phenotype and are unable to achieve a tolerant phenotype upon exposure to LPS. As a result, exposure to pathogens results in a hyper-responsiveness, which could contribute to a continuous inflammatory state. Seahorse assays were conducted to assess the potential effect of cART on metabolic activity. We found that metabolic activity increased when control HIV⁻ cells were treated with cART. This increase resembled PLWH. Such an increased metabolic rate in PLWH can be related to an activation state of these cells. This hyper-responsive trained phenotype is associated with metabolic reprogramming. Monocytic immune cells in a constantly active state require additional energy, which could potentially explain the increase in glycolytic and respiratory rates observed.

## Conclusion

Altogether, data suggest that cART could be responsible for the increased metabolism in terms of respiratory and glycolytic rates observed in monocytes from PLWH. Future experiments are aimed at understanding the role of IKAROS in the cART-induced dysfunctional responses in monocytes.

## Acknowledgments

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