Bioenergetic Health Index: A potential biomarker of the response to TB-HIV patients to anti-TB treatment

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Abstract

Bioenergetics has become central to understanding the pathology of human diseases such as neurodegeneration, diabetes, cancer and cardiovascular diseases. Recent studies have proposed the use of a "Bioenergetic Health Index (BHI)" calculated from extracellular flux analysis can be used as a biomarker to assess patient health with prognostic and diagnostic value[1,2]. Tuberculosis (TB) is responsible for the most co-morbidity of HIV infection in South Africa. We test the hypothesis that the BHI of peripheral circulating monocytes and lymphocytes can be used as a biomarker to assess response to anti-TB treatment in TB/TB-HIV coinfected patients.

We examined the BHI of cells from the peripheral circulation of TB patients enrolled into the Regional Prospective Observational Research in Tuberculosis (RePORT)-study being conducted in Durban, South Africa. Blood was drawn from TB patients at enrolment, two months and six months post frontline TB treatment. Monocytes and lymphocytes were isolated from the peripheral blood mononuclear cells obtained from the patient blood samples. Mitochondrial bioenergetics were analysed using an extracellular flux analyser, and the respiratory parameters (Spare Respiratory Capacity [SRC], ATP Production, non-mitochondrial respiration and proton leak) calculated from the data were used to determine the BHI.

We found that the SRC of lymphocytes, but not monocytes, from patients following treatment was significantly increased. However, no significant increase was observed in BHI of any of the cell populations. Currently, this study is still ongoing to increase patient numbers. The BHI and SRC of these patients will be correlated to sputum smear status during treatment, HIV status and with other clinical parameters, such as diabetes, smoking and age. We conclude that SRC has promising potential as a peripheral biomarker to monitor response to anti-TB treatment.

Methods

- Blood was drawn and PBMC's were isolated from TB-HIV infected patients at time of enrolment and after 2 months and 6 months of TB-treatment.
- Human monocytes and lymphocytes were isolated from PBMC's using Magnetic Activated Cell Sorting (MACS).
- The Seahorse XF96 (extracellular flux analyzer) was used to measure the Oxygen Consumption Rate (OCR) of monocytes and lymphocytes.
- OCR is a measure of oxidative phosphorylation (energy production via electron transport chain [ETC]).
- Inhibitors of the ETC were used to assess various respiratory parameters of the immune cells.

$$BHI = \log \frac{(\text{Spare Respiratory capacity}) \times (\text{ATP production})}{(\text{Non} - \text{Mitochondrial Respiration}) \times (\text{Proton leak})}$$
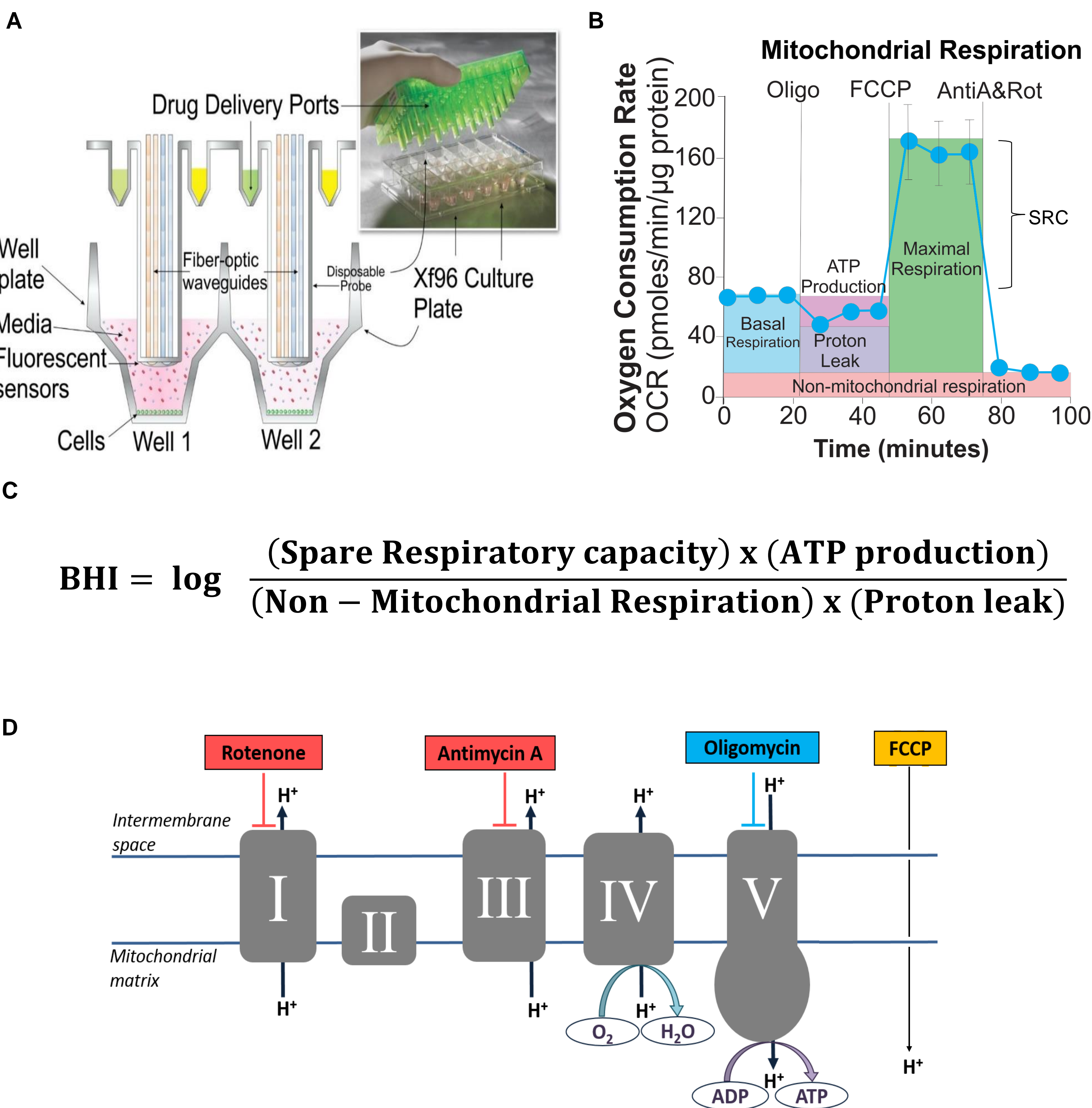
Figure 1: (A) Seahorse XF96 probes measuring extracellular flux, (B) mitochondrial respiration profile illustrating the respiratory parameters used to calculate BHI, (C) equation used to calculate BHI from the respiratory parameters[1], (D) electron transport chain and target sites of respiratory modulators.
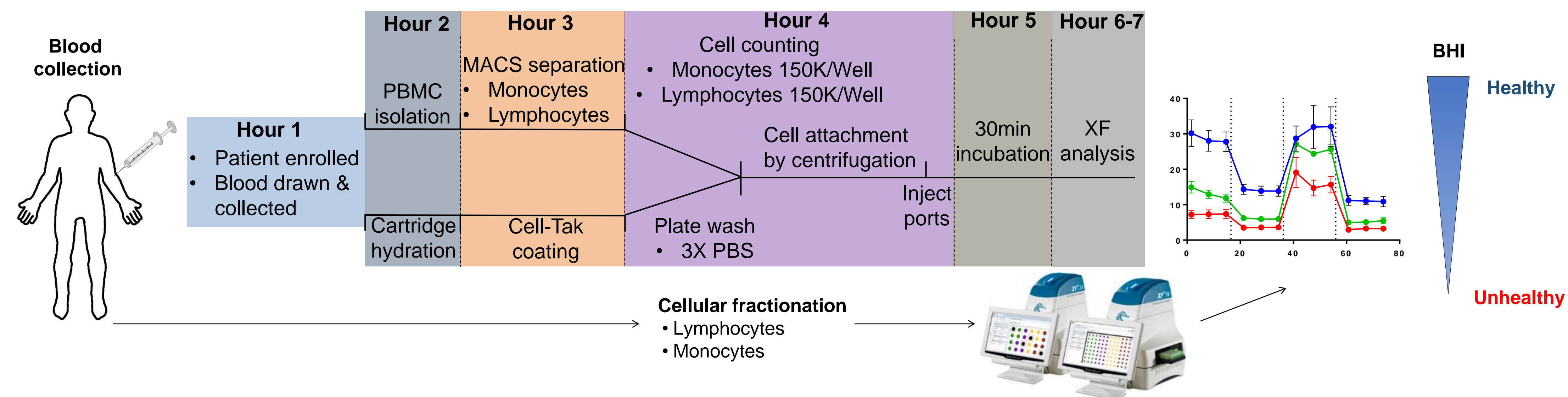

Figure 2: Workflow to assess the bioenergetic parameters of monocytes and lymphocytes to determine the BHI.

Results

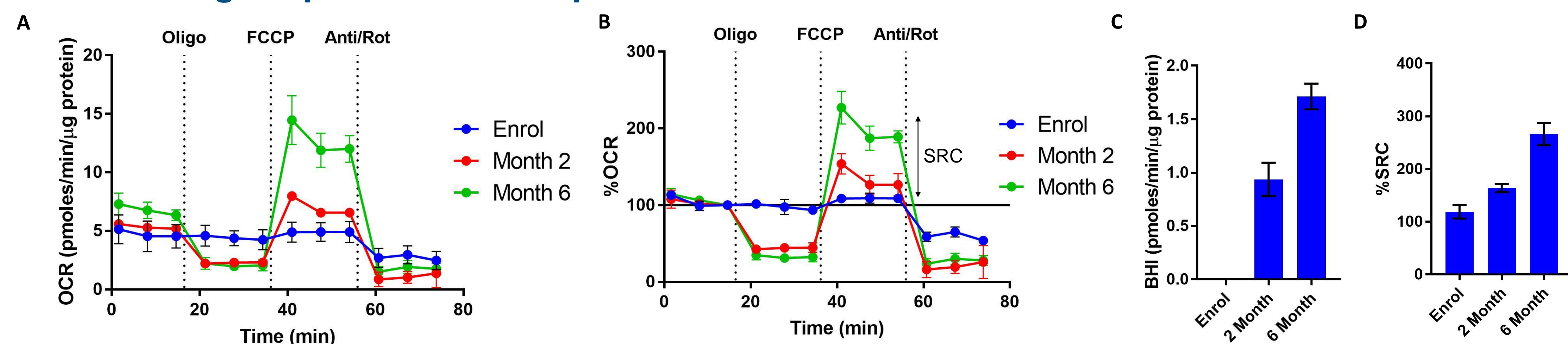### Bioenergetic profiles show improvement from enrolment to 6 months

Figure 3: OCR (A) and baselined OCR profile (B) of lymphocytes from a selected patient at enrolment, Month 2 and Month 6 of anti-TB treatment. (C) BHI and (D) %SRC of selected patient at enrolment, 2 and 6 month of anti-TB treatment.

Lymphocyte Spare Respiratory Capacity improves after 6 months of anti-TB treatment

- Due to the current low numbers of patients that have completed 6 months of treatment in this study, no significant changes were observed in the BHI of the monocytes and lymphocytes during treatment.
- SRC of lymphocytes showed a significant improvement after 6 months of anti-TB treatment as seen in both HIV+ and HIV- patients.
- In some patients, lower BHI and SRC values were seen at 2 months of treatment. This could be due to the intense anti-TB drug regimen for the first two months having an effect on the mitochondrial bioenergetics of these cell populations.
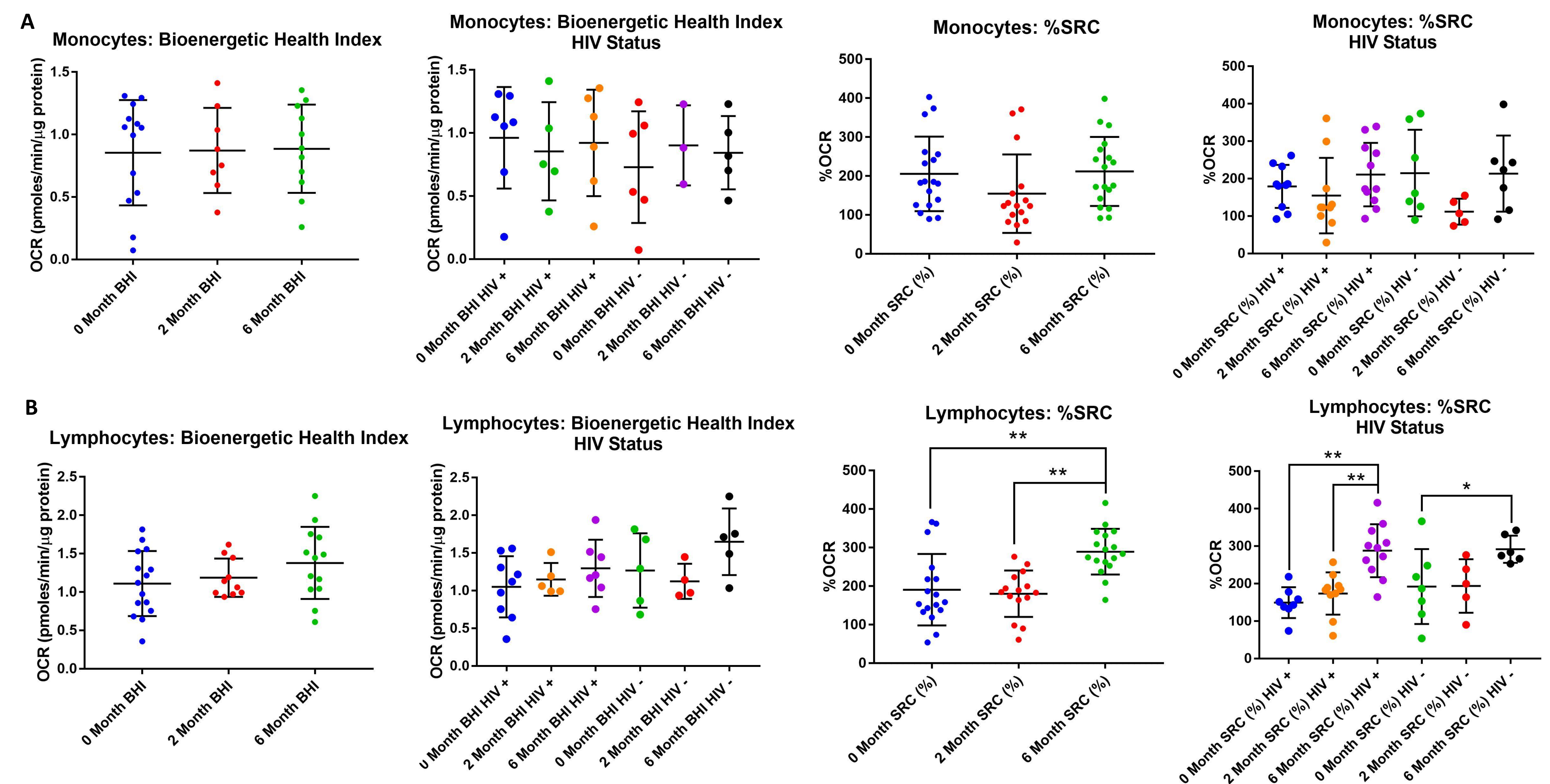

Figure 4: BHI and %SRC of monocytes (A) and lymphocytes (B) isolated from the blood of TB-HIV patients at enrolment (0 month) and at 2 month and 6 month follow-ups after starting anti-TB treatment. Unless indicated, no significant differences were found. * ($p \le 0.05$), ** ($p \le 0.01$).

Conclusions

- SRC of lymphocytes has been shown to significantly improve after 6 months of TB treatment in both HIV+ and HIV- patients.
- The BHI of monocytes and lymphocytes from TB-HIV patients did not significantly improve. This is probably due to the low numbers of 6 month follow-up patients.
- Further correlations between bioenergetic parameters and other clinical parameters are currently being determined as the study is ongoing.

References:

1. Chacko et al., 2014. The Bioenergetic Health Index: a new concept in mitochondrial translational research. *Clin. Sci.* Vol. 127, pp. 367-373.

2. Kramer et al., 2015. Decreased Bioenergetic Health Index in monocytes isolated from the pericardial fluid and blood of post-operative cardiac surgery patients. *Biosci. Rep.* Vol. 35, art:e00237.