Application Note

Assessing functional metabolism using cells sourced from blood

Real-time, kinetic measurements link mitochondrial function to disease diagnosis and progression.

RESEARCH AREAS

Neurodegeneration
Asthma
Obesity, Diabetes, & Metabolic Disorders
Aging
Cardiovascular
Cancer
Immunology

ASSAY TYPE

Seahorse XF Cell Mito Stress Test
Seahorse XF Glycolysis Stress Test
Seahores XF Cell Energy Phenotype Test

KEYWORDS

BHI, PBMCs, platelets, mitochondrial function, glycolysis, mitochondrial respiration

Recent studies have linked mitochondrial dysfunction to pathologies such as cancer, diabetes, and cardiovascular disease. However, a lack of relevant cellular models impedes studies of these pathologies. Obtaining patient samples usually involves a high degree of invasiveness, and furthermore these samples may not be a sufficient indicator of mitochondrial dysfunction. Alternatively, blood cells or platelets could provide an advantageous option to tissue samples as an accessible source of mitochondria.

Peripheral blood mononuclear cells (PBMCs) are a heterogeneous cell population, comprised of several cell types including monocytes, T cells, and natural killer cells. It has been suggested that this cell population mirrors systemic changes within the body and would, for this reason, provide a source of sensitive biomarkers (Chacko *et al.*, 2014; Maynard *et al.*, 2013). Consequently, researchers are increasingly exploring these cells as indices of diagnosis and disease monitoring. For example, Seahorse XF technology has been used with PBMCs to explore the condensing of a patient's mitochondrial respiration profile into a single value, termed the Bioenergetic Health Index (BHI), which may be used as a predictive biomarker.
This application note describes three recent publications that utilized Seahorse XF technology to measure the functional metabolism of blood cells and platelets derived from normal and affected patients.

In a study by Czajka *et al.* (2015), the authors employed PBMCs in a cross-sectional study comparing patients with diabetic nephropathy (DN), diabetic patients without kidney disease (DC), and healthy controls (HC). DN is the most common cause of chronic renal failure, with the number of cases rising in proportion to the number of diabetic patients.

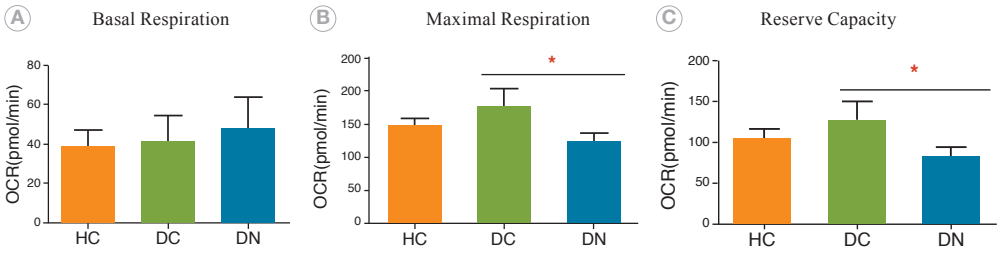

Figure 1 | Analysis of mitochondrial respiration in PBMCs.

PBMCs isolated from patients with diabetes nephropathy (DN); diabetic patients (DC); and healthy controls (HC). Basal (A), maximal respiration (B), and reserve capacity (C) were calculated following the sequential addition of oligomycin, FCCP, and a combination of antimycin A and rotenone.

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The authors hypothesized that assessing OCR and ECAR values in PBMCs would provide an indication of metabolic stress associated with diabetes. Using Seahorse XF technology, they determined the metabolic profiles of PBMCs derived from each of tested groups: DN, DC, and HC. As shown in Figure 1, the metabolic parameters of each group revealed significant differences. While the basal respiration rates appeared to be similar amongst all three groups (Figure 1A), both maximal respiration and reserve capacity (Figures 1B and 1C, respectively) were significantly reduced in DN patients.

These data indicate that under normal conditions, basal mitochondrial respiration occurs similarly in each of the three tested groups. However, under stress, PBMCs-derived from DN patients exhibited reduced activity. The authors used an Seahores XF^{e}96 Extracellular Flux Analyzer, together with the Seahorse XF Cell Mito Stress Test to examine freshly isolated human PBMCs. The authors' analyses demonstrate a distinct difference in the functional metabolism of PBMCs isolated from DN, DC, and HC individuals, and further suggest that the reduced mitochondrial metabolism may lead to DN progression. This study highlights the use of PBMCs as a model of metabolic dysfunction in patients with DN, and may be developed as a translational indicator of mitochondrial function.

Discussion

Researchers are using Seahorse XF technology, in combination with XF stress tests, consumables, and reagents to uncover relevant functional metabolic data in a variety of therapeutic areas. As described in these publications, blood cells and platelets are capable of revealing tissue functionality without the need for highly invasive procedures.

Aging presents a universal and complex phenomenon, characterized by progressive changes in various physiological functions. While physical testing of an individual or group integrates several physiological systems, such as the musculoskeletal system, these tests neglect the biological mechanisms that are associated with the aging process, and do not reveal the functional aspects of a specific tissue. In study by Tyrrell *et al*. (2015), the authors used human PBMCs isolated from community-dwelling, sedentary, overweight, or obese males to determine if there is a relationship between the metabolic signature of PBMCs and physical function.

The authors examined PBMCs using an Seahorse XF24-3 Extracellular Flux Analyzer in conjunction with the Seahorse XF Cell Mito Stress Test. They observed that PBMCs exhibited increased maximal respiration and spare respiratory capacity were associated with greater leg muscle quality, grip strength, and lower levels of IL-6, a cytokine known to play a significant role in chronic inflammation. These results indicate a correlation between the metabolic profiles of PBMCs and aging markers, including muscle strength and quality, and overall physical function. As noted by the authors, assessing PBMCs may provide a diagnostic and prognostic tool that can be readily utilized in clinical trials, as well as gerontology.

In any research area, utilizing a relevant cell model to study metabolic changes is crucial to designing effective disease-specific therapeutics and treatment strategies. For example, altered cell metabolism has been shown to play a role in asthma, a chronic inflammatory disease. However, changes in either mitochondrial respiration or glycolysis have yet to be detected in circulating cells isolated from asthma patients. In a study by Xu *et al*. (2015), the authors examined the use of human platelets to screen patients affected by asthma.

Using an Seahorse XF24 Extracellular Flux Analyzer the authors assessed platelets isolated from venous blood samples. They observed that asthmatic patient-derived platelets are more oxidative, and, therefore, exhibited a reduced reliance on glycolysis, as compared to the metabolic profile of healthy controls. This metabolic shift from glycolysis to mitochondrial respiration in asthmatic patients provides new insights into the metabolic characteristics of asthma, and further highlights the use of blood cells as a biomarker to monitor disease severity.

Materials and Methods

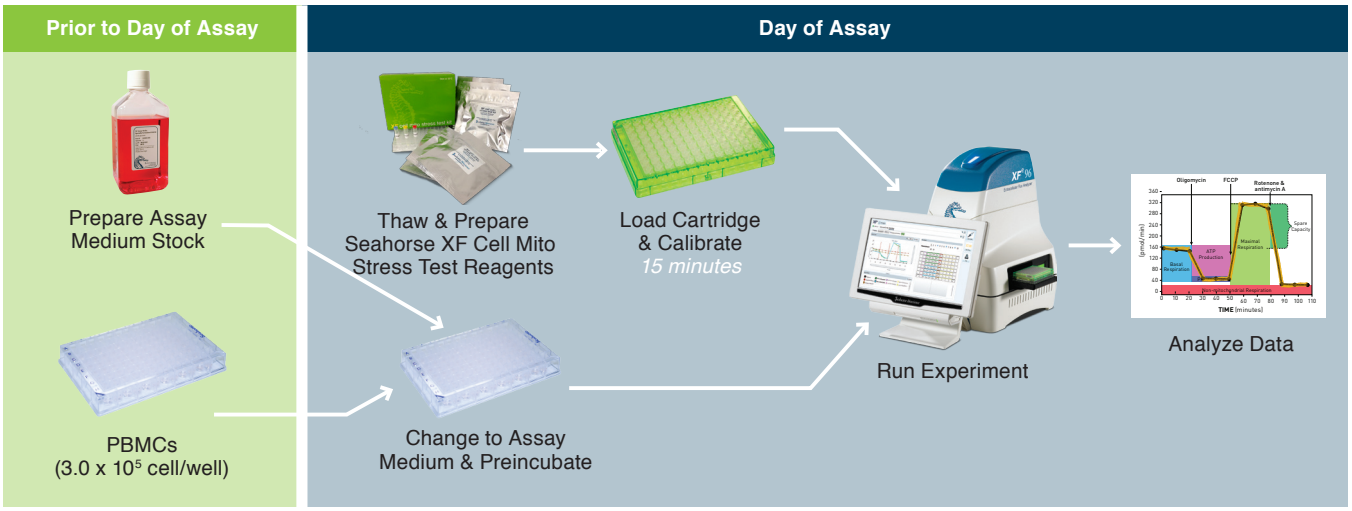
PBMCs were isolated from human volunteers diagnosed with diabetes, either with diabetic nephropathy or normal renal function, and healthy controls. Cells were then seeded at a density of 3.0 x10^5 cells/well prior to analysis.

XF Bioenergetic Analysis

Metabolic analyses were performed using an Seahorse XF^{e}96 Extracellular Flux Analyzer which enables real-time, simultaneous measurements of the oxygen consumption rate (OCR), and the extracellular acidification rate (ECAR), via a transient microchamber within each well of specialized cell culture microplates.

As shown in Figure 2, PBMCs were seeded in each well of an Seahorse XF^{e}96 Cell Culture Microplate. For the Seahorse XF Cell Mito Stress Test, cells were sequentially injected with oligomycin, FCCP, and a combination of antimycin A and rotenone. Data are presented as mean ± SEM and analyzed by one-way ANOVA with Tukey's test.

Figure 2 | Flow Chart of XF Assay



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