Measuring Functional Metabolism of Brite Adipocytes

Mitochondrial Function Correlates to Brown Adipocyte Function

Application Brief

Introduction

As the obesity and type 2 diabetes epidemic continues to worsen, research into therapies to mitigate symptoms has intensified. One candidate receiving increased attention is the development of brown adipose tissue (BAT). BAT functions in metabolizing fat and generating heat for non-shivering thermogenesis, an activity that can contribute to overall metabolic rate, which makes BAT an attractive therapeutic target. Studies have shown that BAT is not found in great abundance in adults, therefore generating cells that have the same functional characteristics as brown adipocytes may offer benefits in the treatment of obesity and diabetes. Methods for generating brown adipocyte-like cells include using white adipocytes that express thermogenic capabilities (termed beige-in-white or brite adipocytes) that undergo a process called browning. This Application Brief describes the use of Agilent Seahorse XF technology in the study of these brown adipocyte-like cells for obesity and diabetes research.

During BAT development, specific gene programs such as peroxisome proliferator-activated receptor γ (PPARγ), a known regulator of adipocyte differentiation, are upregulated. PPARγ controls gene expression for key adipocyte functions, including lipid transport and metabolism, insulin signaling, and adipocyte proliferation.

Loft; *et al.* (2015)[1] used Agilent Seahorse XF technology in conjunction with a genomics-wide approach to understand the mechanism involved in adipocyte browning. Using human multipotent adipose-derived stem cells (hMADs), the authors demonstrate a connection between brite adipocyte generation, genetic programming, and PPARγ.

Agilent Technologies

The authors first examined the effect of rosiglitazone, a known agonist of PPARγ, on white and brite adipocytes. From day 13 through either day 16 or 19, hMADs were cultured in the presence of rosiglitazone to induce browning, and then examined using the Agilent Seahorse XF Cell Mito Stress Test. Figure 1A shows that the metabolic signatures of white and brite adipocytes are significantly different when rosiglitazone is omitted from the brite adipocyte media at day 16. The observation that brite adipocytes maintain increased maximal, uncoupled, and basal respiration from days 16 through 19 indicates a commitment by the brite-selective genetic program to the metabolic properties of brite adipocytes.

To further assess the effects of rosiglitazone and KLF11, a transcription factor involved in browning, the authors used hMADs adipocytes transduced with shRNA vectors to knockdown KLF11 and then subsequently treated with either DMSO or rosiglitazone. Figure 1B shows that rosiglitazone was unable to induce a higher oxygen consumption rate (OCR) following KLF11 knockdown. These data indicate that the browning induced by rosiglitazone is dependent on KLF11, and that the process of browning, that is the generation of brite adipocytes, links a genetic program and mitochondrial respiration. In this study, the authors used an Agilent Seahorse XF24 Extracellular Flux Analyzer along with the Agilent Seahorse XF Cell Mito Stress Test to determine the effects of genetic programming on mitochondrial respiration. Their analyses elucidated a possible mechanism underlying brite adipocyte development.

Results and Discussion

In assessing brite adipocytes, the beneficial capabilities of brown adipocytes must be maintained. One characteristic of brown adipocytes in BAT is the presence of uncoupling protein UCP1, which catalyzes proton leak, generating heat at the expense of ATP production (Divakaruni and Brand, 2011)[2]. Bartesaghi; *et al.* (2015)[3] focused their study on human adipose-derived stromal/progenitor cells (hASCs) to determine whether these cells can be successfully converted into thermogenic beige adipocytes and maintain comparable functionality to brown adipocytes.

The authors used a Agilent Seahorse XF24 Analyzer to determine the metabolic characteristics in hASC-derived brite adipocytes. They observed a rosiglitazone-dependent increase in overall mitochondrial respiration, and specifically uncoupling, maximal respiration, and spare respiratory capacity. Further examination illustrated a requirement for UCP1 to stimulate uncoupled respiration in these generated adipocytes. These data demonstrate that human stem cells cultured from white adipose tissue can be differentiated into beige adipocytes, suggesting that this induced differentiation paradigm may be a treatment for obesity, and potentially reduce the effects of metabolic disorders.

There have been many advances in the field of stem cells, in both embryonic and adult stem cells. With the ability to differentiate into different cell types and replicate indefinitely, the use of stem cells to generate brite adipocytes presents a possible fruitful research avenue. Silva; *et al.* (2014)[4] studied stem cells derived from BAT, and observed that brown adipose-derived stem cells (BADSCs) have distinct characteristics, which they suggest may be beneficial in the treatment of obesity and diabetes.

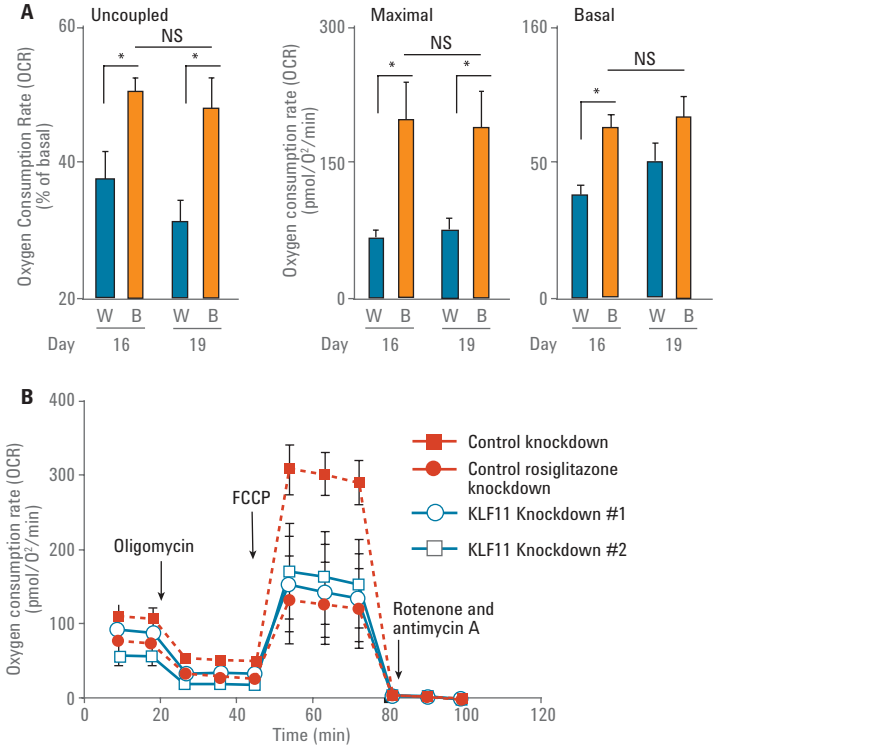

Figure 1. Brite adipocytes have distinct mitochondrial function. A) Analysis of maximal, uncoupled, and basal respiration in both white (W) and brite (B) adipocytes, comparing day 16 and day 19 cultures. B) Agilent Seahorse XF Cell Mito Stress Test profile of hMADs transduced with either control (red traces) or KLF11 knockdown (blue traces) and subsequently treated with DMSO or rosiglitazone.

Materials and Methods

hMADs were cultured in low-glucose DMEM, supplemented with 10 % fetal bovine serum (FBS), HEPES (10 mM), L-glutamine (2 mM), penicillin (62.5 µg/mL), streptomycin (100 µg/mL), and hFGF2 (2.5 ng/mL). Following confluence, cell differentiation was induced using DMEM/Ham's F12, supplemented with transferrin (10 µg/mL), dexamethasone (1 µM), 2-isobutyl-1-methylxanthine (IBMX) (500 µM), insulin (0.85 µM), and T3 (0.2 nM). From day 13 to day 16, cells were treated with either DMSO, to maintain white adipocytes, or 0.5 µM rosiglitazone to induce brite adipocyte generation. Following day 16, rosiglitazone was removed from the growth medium of both white and brite adipocytes.

XF Bioenergetic analysis

Metabolic analyses were performed using an Agilent Seahorse XF24 Analyzer, which enables real-time, simultaneous rate measurement of OCRs and extracellular acidification rates (ECARs) by creating a transient micro chamber within each well of the specialized cell culture microplates.

As shown in Figure 2, hMADs were seeded onto Agilent Seahorse XF24 Cell Culture Microplates, then differentiated. For the Agilent Seahorse XF Cell Mito Stress Test, following basal respiration, the mitochondrial effectors oligomycin, FCCP, and a mixture of rotenone and antimycin A were injected sequentially. Data are shown as mean values ±SEM. A two-tailed Student's t-test was used to calculate statistical significance.

Using Agilent Seahorse XF technology to study the generation of brown adipocyte-like cells reveals distinct metabolic characteristics that provide a connection to functionality and brite adipocyte development.

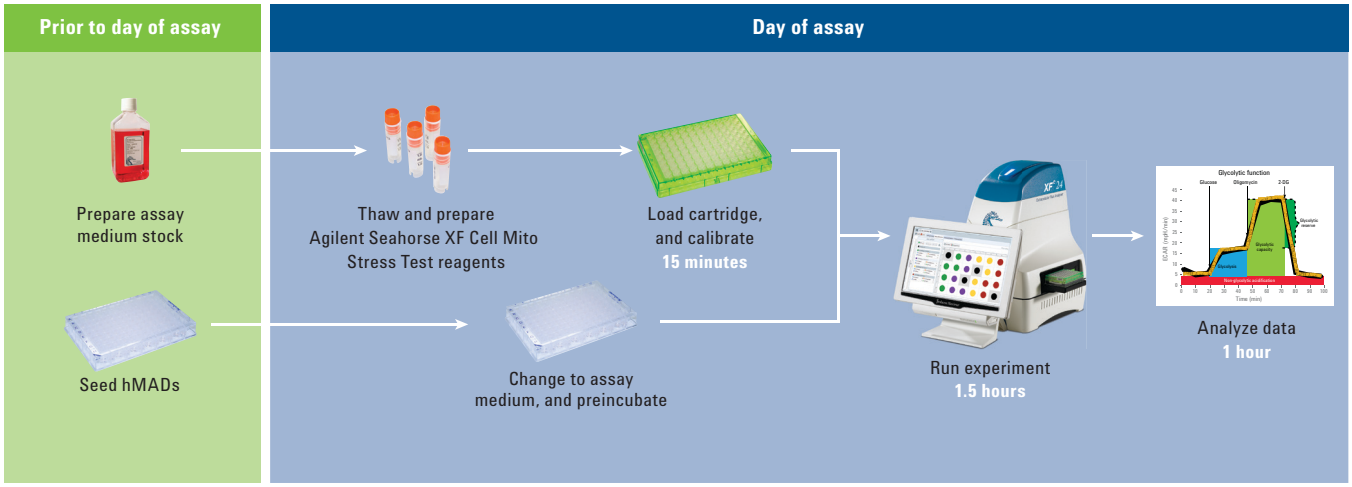

Figure 2. Flow chart of the XF assay.

References

1. Loft; *et al.* Browning of human adipocytes requires KLF11 and reprogramming of PPARγ superenhancers. *Genes Dev*. **2015**, *29(1)*, 7-22.

2. Divakaruni, A. S.; Brand, M. D. The regulation and physiology of mitochondrial proton leak. *Physiology* **2011**, *26(3)*, 192-205.

3. Bartesaghi; *et al.* Thermogenic activity of UCP1 in human white fat-derived beige adipocytes. *Mol. Endocrinol.* **2015**, *29(1)*, 130-9.

4. Silva; *et al.* Metabolically active human brown adipose tissue derived stem cells. *Stem cells*. **2014**, *32(2)*, 572-81.

www.agilent.com

Published in the USA, December 1, 2016
5991-7133EN

Agilent Technologies