Optimized workflow to assess metabolic profile during adipocyte differentiation and in adipocyte tissue

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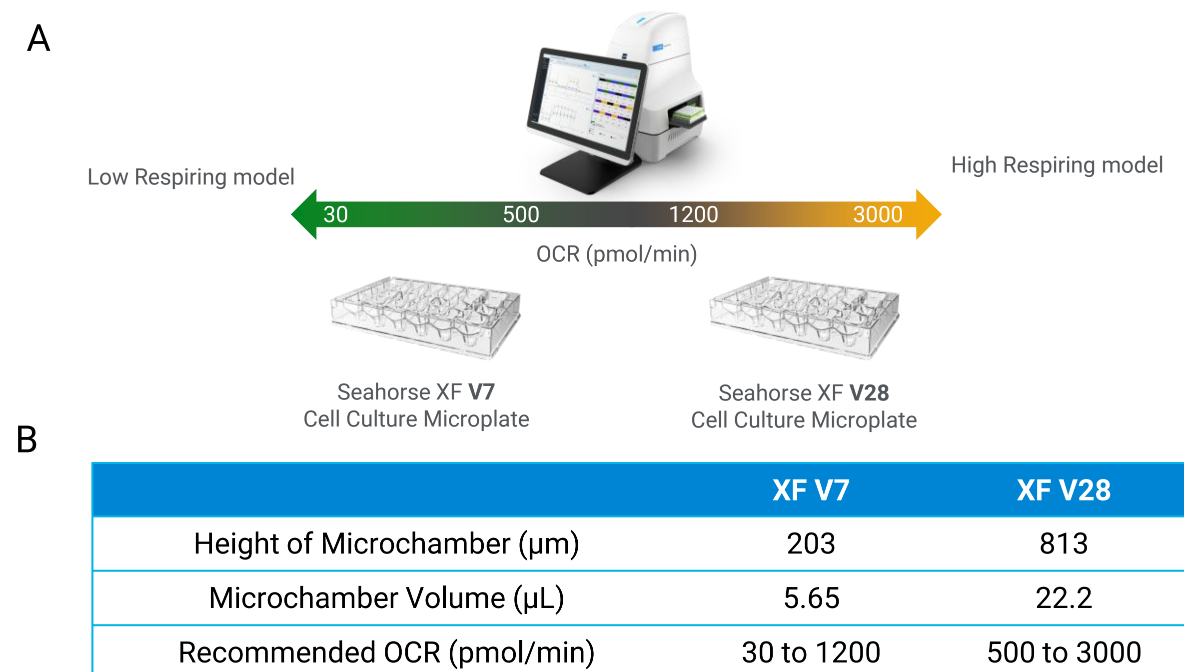
Introduction

In adipocytes, mitochondrial dysfunction is associated with metabolic disorders such as obesity and type 2 diabetes. During adipocyte differentiation, mitochondrial activity adjusts to meet evolving metabolic demands. While Agilent Seahorse XF analyzers are commonly used for in vitro mitochondrial function assessments, monitoring metabolic phenotypes in mature adipocytes is challenging due to the significant increase in metabolic rates during differentiation. In this study, we developed an optimized workflow for accurate metabolic profiling during in vitro differentiation by combining the standard Agilent Seahorse XFe24/XF Flex V7 PS Culture Microplate with the Agilent Seahorse XFe24/XF Flex V28 Culture Microplate and with the Seahorse XF Flex Analyzer. This expands the detection range of the instrument and captures a broad range of metabolic activities across several differentiation stages with great precision and accuracy. In addition, the instrument is compatible with new consumables for metabolic measurements in tissue samples and organoid-models and allows extending bioenergetics research to ex vivo and in vitro 3D models.

Experimental

Combined usage of XFV7 and V28 microplate in XF Flex Analyzer



	XF V7	XF V28
Height of Microchamber (µm)	203	813
Microchamber Volume (µL)	5.65	22.2
Recommended OCR (pmol/min)	30 to 1200	500 to 3000

Figure 1: (A) Schematic diagram showing extended detection range in the Seahorse XF Flex Analyzer by combining XF V7 and XF V28 Microplates. (B) Table showing the difference between Seahorse XF V7 and XF V28 cell culture microplates.

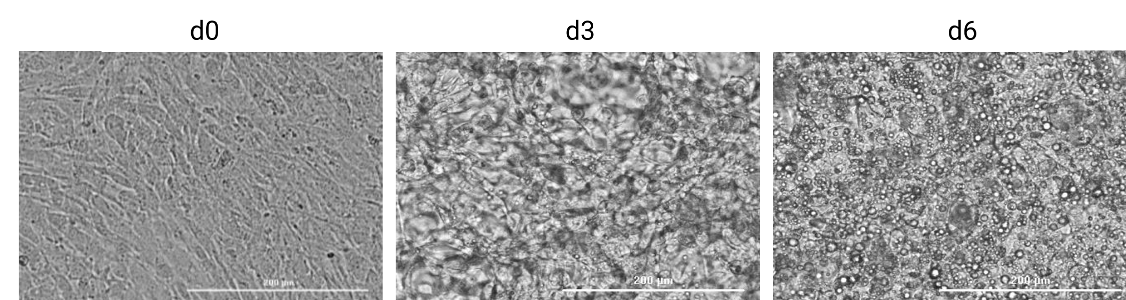


Figure 2: Phase contrast images (10x magnification) of 3T3-L1 cells two days post confluent and before differentiation (d0), 3 days (d3) and 6 days (d6) after differentiation, acquired using the Agilent BioTek Cytation 5 Cell Imaging Multimode Reader. Scale bar = 200 µm. Pre-confluent 3T3-L1 cells were cultured in DMEM with 10% Calf Bovine Serum, while differentiation was induced on d0 using DMEM supplemented with 10% FBS, 0.5mM IBMX, 5 µM Rosiglitazone, 1 µM dexamethasone, and 200 nM insulin. Two days after differentiation induction, media was replaced with DMEM containing 10% FBS and 200 nM insulin. From day 4 onward, cells were maintained in differentiation media consisting of DMEM with 10% FBS and 20 nM insulin, with media refreshed every 1 to 2 days

Results and Discussion

Determination of the limits of the XF V7 microplate OCR detection range

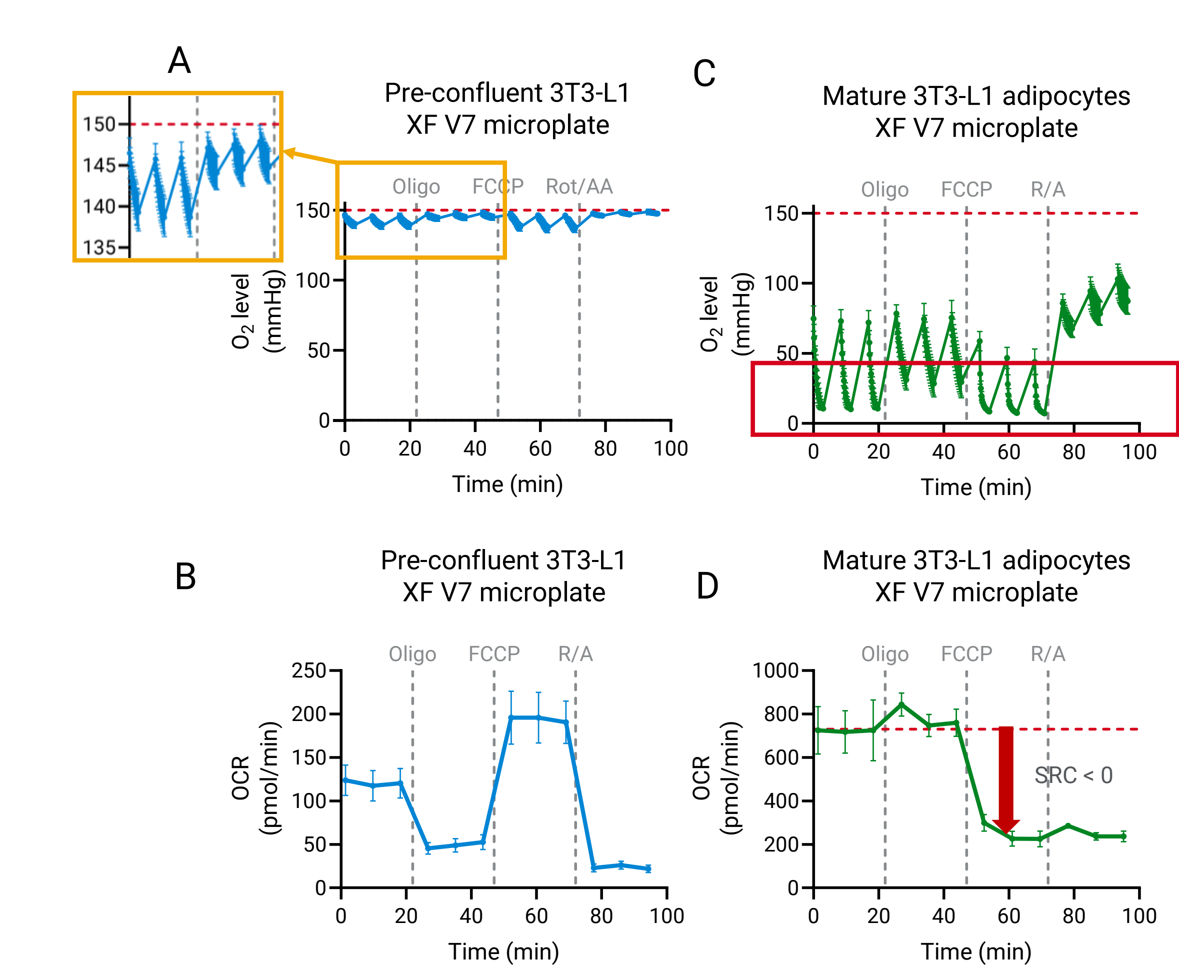
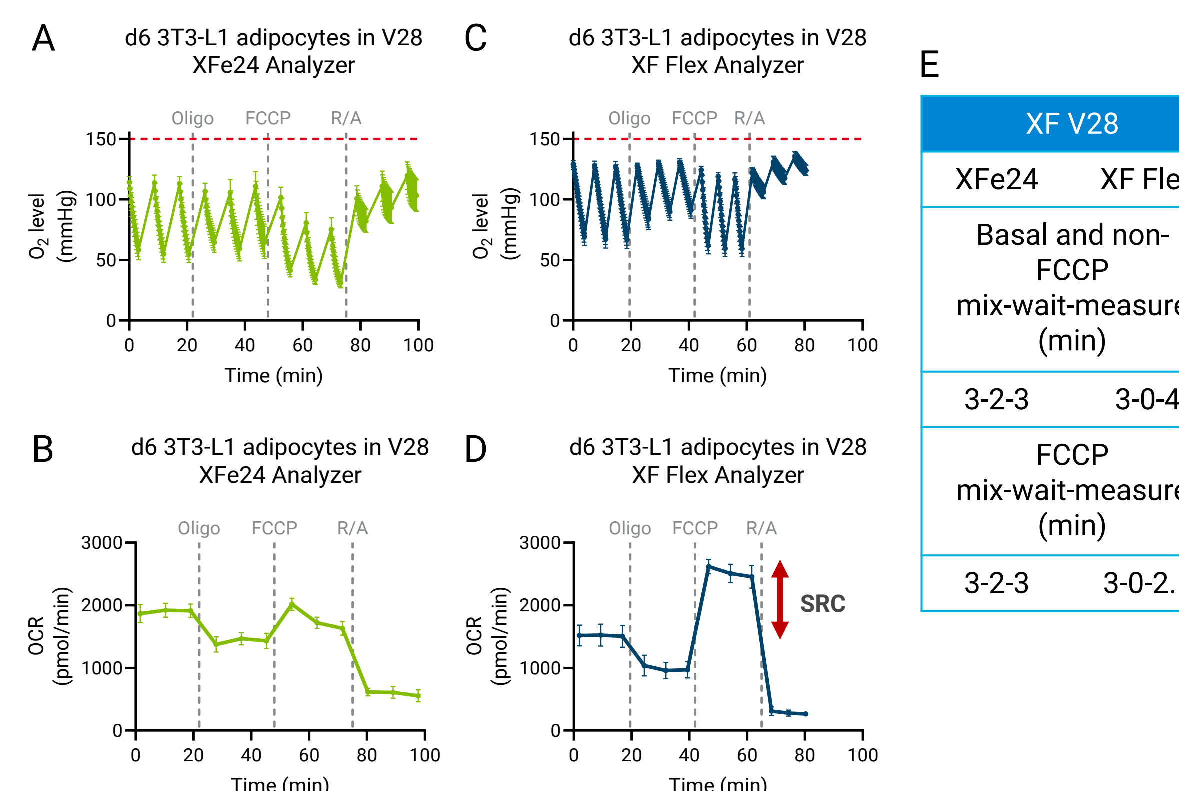


Figure 3: XF Cell Mito Stress test comparison between pre-confluent 3T3-L1 fibroblasts (A, B) and mature adipocytes (C, D) using the XF V7 microplate and the Seahorse XF Flex Analyzer following the default protocol of 3 min mix, 2 min wait and 3 min measurement. Mature adipocytes' high respiration results in severe oxygen depletion during measurements (red box) and underestimation of maximal respiration (C, D), resulting in false negative spare respiratory capacity calculations (red arrow). 2.5 µM Oligomycin (Oligo), 1 µM Rotenone/Antimycin A (R/A), and optimized concentration of FCCP for each differentiation stage were used throughout this experiment, that is, 1.5 µM for pre-confluent cells and 2 µM for d0 and after.

Improved OCR measurements for high-respiring cells using the XF V28 microplate and the Seahorse XF Flex Analyzer



XF V28	
XFe24	XF Flex
Basal and non-FCCP mix-wait-measure (min)	
3-2-3	3-0-4
FCCP mix-wait-measure (min)	
3-2-3	3-0-2.5

Figure 4: Comparison of XF Cell Mito Stress Test results in mature 3T3-L1 adipocytes (day 6) using the XFe24 Analyzer (A–B) and XF Flex analyzer (C–D) with the XF V28 microplate. The optimized mixing program and protocol (E) for the XF V28 microplate, implemented in the XF Flex analyzer, enables oxygen recovery between measurements and accurate determination of maximal respiration and spare respiratory capacity (SRC; red arrow).

Combining XF V7 and XF V28 microplate assays for accurate metabolic profiling across adipocyte differentiation stages

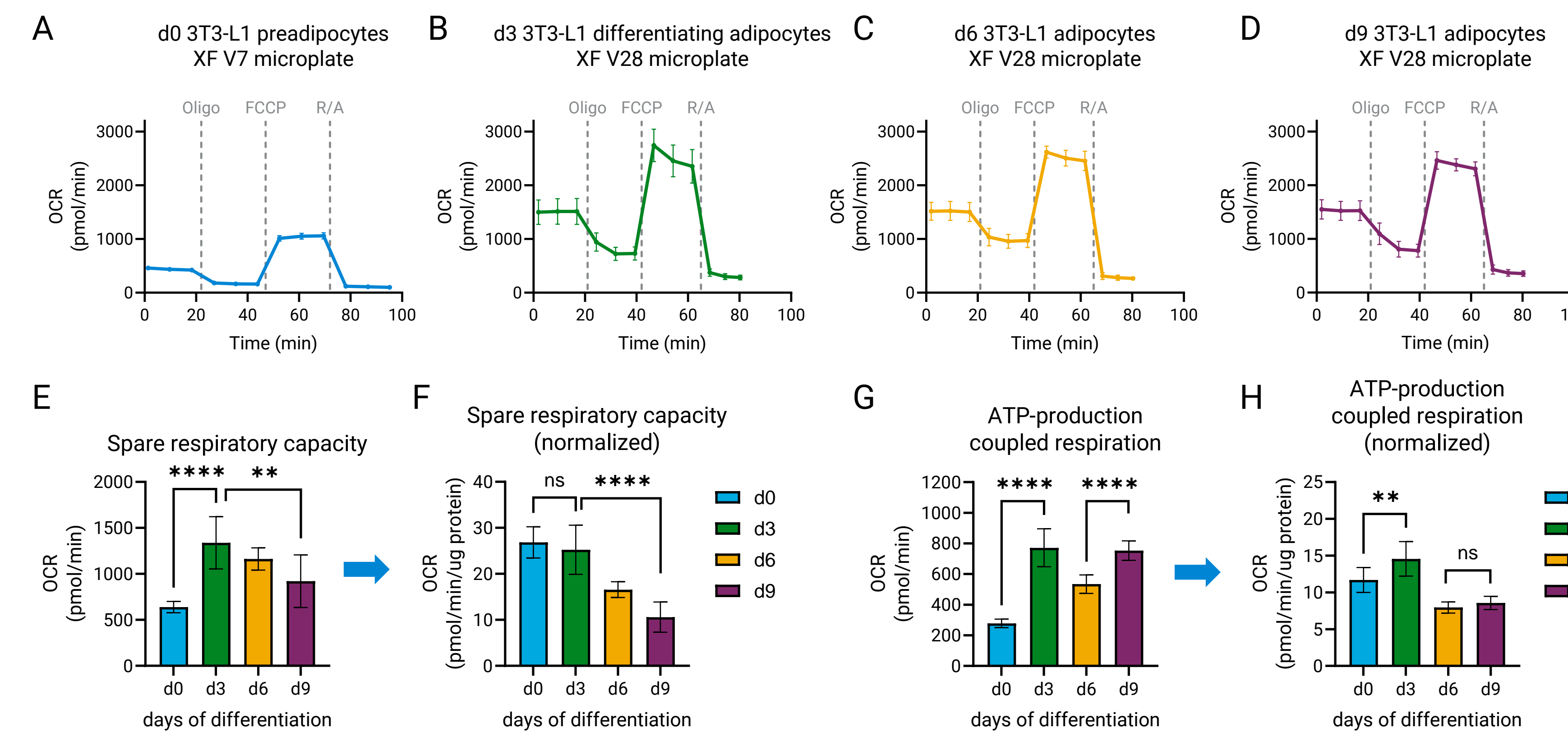


Figure 5: XF Cell Mito Stress Test analysis of 3T3-L1 cells during differentiation using a combination of XF V7 and XF V28 microplates on the XF Flex analyzer. (A–D) OCR kinetic profiles of d0 preadipocytes (A), d3 differentiating cells (B), and mature adipocytes at d6 (C) and d9 (D). Spare respiratory capacity (E, F) and ATP-production coupled respiration (G, H) across different stages of differentiation, not normalized (E, G) or normalized to protein content (F, H). Data represents mean ± SD of 10 technical replicates. **P<0.01, ****p<0.0001.

Substrate oxidation stress test reveal differential fuel dependence in pre-and mature 3T3-L1 adipocytes

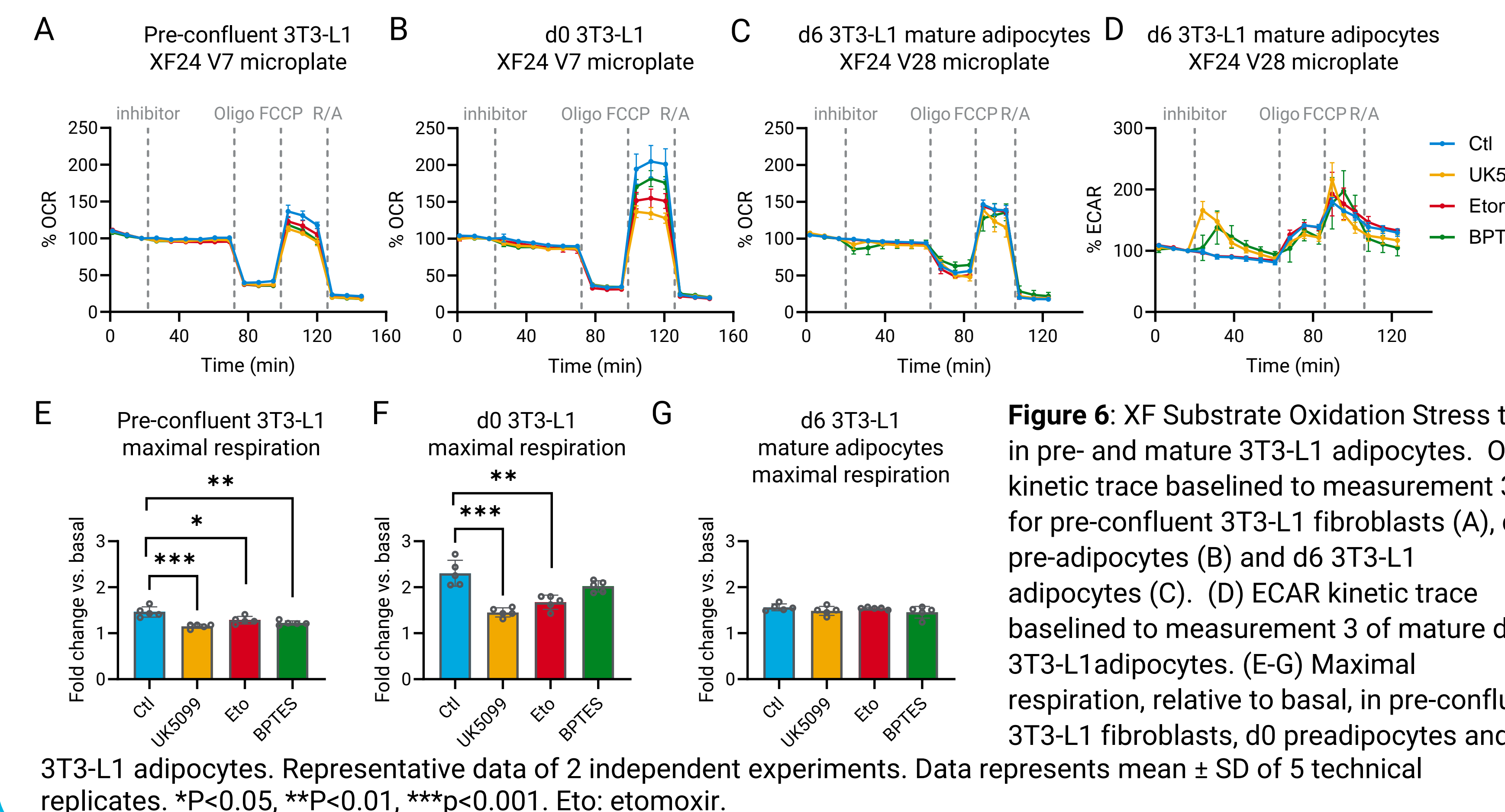
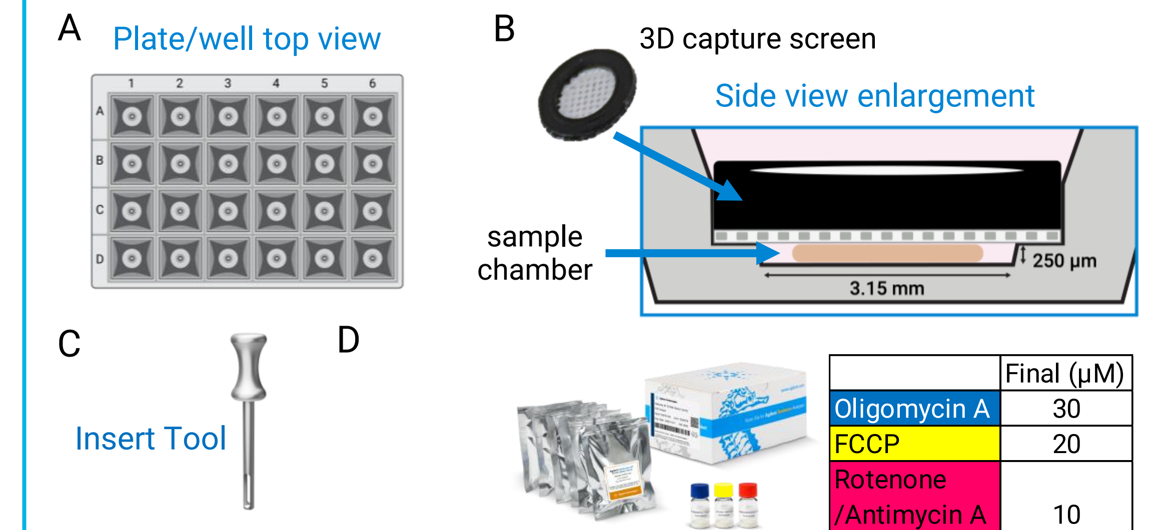


Figure 6: XF Substrate Oxidation Stress test in pre- and mature 3T3-L1 adipocytes. OCR kinetic trace baselined to measurement 3 for pre-confluent 3T3-L1 fibroblasts (A), d0 pre-adipocytes (B) and d6 3T3-L1 adipocytes (C). (D) ECAR kinetic trace baselined to measurement 3 of mature d6 3T3-L1adipocytes. (E-G) Maximal respiration, relative to basal, in pre-confluent 3T3-L1 fibroblasts, d0 preadipocytes and d6 3T3-L1 adipocytes. Representative data of 2 independent experiments. Data represents mean ± SD of 5 technical replicates. *P<0.05, **P<0.01, ***p<0.001. Eto: etomoxir.

Conclusions

- Combining the XF V7 and XF V28 microplate extends the detection range of metabolic characterization using the Agilent Seahorse XF Flex analyzer allowing the study of metabolic profile of a wider variety of cell types.
- We provided a workflow for accurate assessment of mitochondrial function during the whole course of adipocyte differentiation, reducing the need to adjust differentiation protocols or timeframes to accommodate precise metabolic measurements and delivering new insights into cell metabolism and its modulation.
- XF Flex tissue workflow and consumables provide the necessary sensitivity to gain robust tissue real-time respiratory responses to mitochondrial modulators.

New XF Flex dedicated consumables for tissue metabolic studies



	Final (µM)
Oligomycin A	30
FCCP	20
Rotenone /Antimycin A	10

Figure 7: (A) Top view and (B) side view of XF Flex 3D capture microplate (L-plate). (C) Insert tool for placing the 3D capture screen. (D) XF 3D Mito Stress Test kit, ready to use and with optimized concentration for tissues.

Metabolic profiling of white and brown adipose tissues

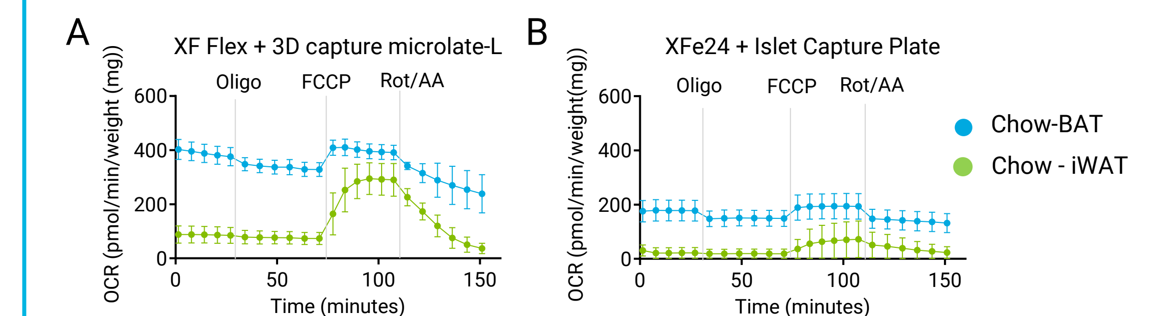


Figure 8: (A) OCR kinetic trace of XF 3D Mito Stress Test of interscapular brown adipose tissue (BAT) and inguinal white adipose tissue (iWAT) from 10 weeks old C57BL/6j chow fed mice performed in XF Flex analyzer using 3D capture microplate-L. (B) OCR kinetic trace of the XF 3D Mito Stress Test of the same tissue parallelly assayed in XFe24 analyzer using islet capture plate.

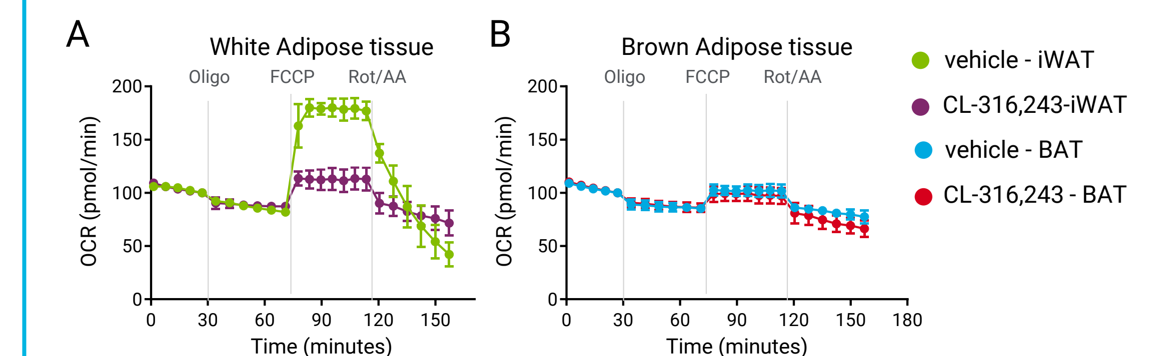


Figure 9: XF 3D Mito Stress Test performed using the 3D capture microplate-L in XF Flex analyzer. Chow fed mice were injected with CL-316,243 (a β3-adrenergic receptor agonist, 1mg/kg) for 7 days before tissues were harvest for XF assays. OCR kinetic trace (Baselined to measurement 5) of iWAT (A) and BAT (B) from mice receiving vehicle or CL-316,243.

References

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2. A superior system for real-time metabolic analysis with brain tissue and other 3D models. https://www.agilent.com/cs/library/applications/an-superior-system-for-real-time-metabolic-analysis-5994-8309EN-agilent.pdf

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