Poster Reprint

ASMS 2026
ThP 210

A Holistic View of GLP 1R Agonist–Mediated Cellular Response Revealed Through Orthogonal Technologies

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Introduction

Glucagon-like peptide-1 receptor (GLP1R) agonists have gained significant prominence due to their use in treating type 2 diabetes and obesity, driving increased research interest in their broader biological actions.

From a drug development perspective, this integrated cellular profiling approach supports translational evaluation of GLP1R agonists by linking receptor activation to downstream metabolic and bioenergetic responses relevant to drug efficacy and safety.

Thus, understanding how these compounds influence cellular systems, including potential off-target effects, is important for interpreting their wider impact as proxies for physiological responses.

Here we combine multiple cell analysis techniques, including bioenergetic analysis, fluorescence imaging, receptor activation assay, and MS-based metabolomics and lipidomics to investigate the cellular response to GLP1R agonists under different growth conditions.

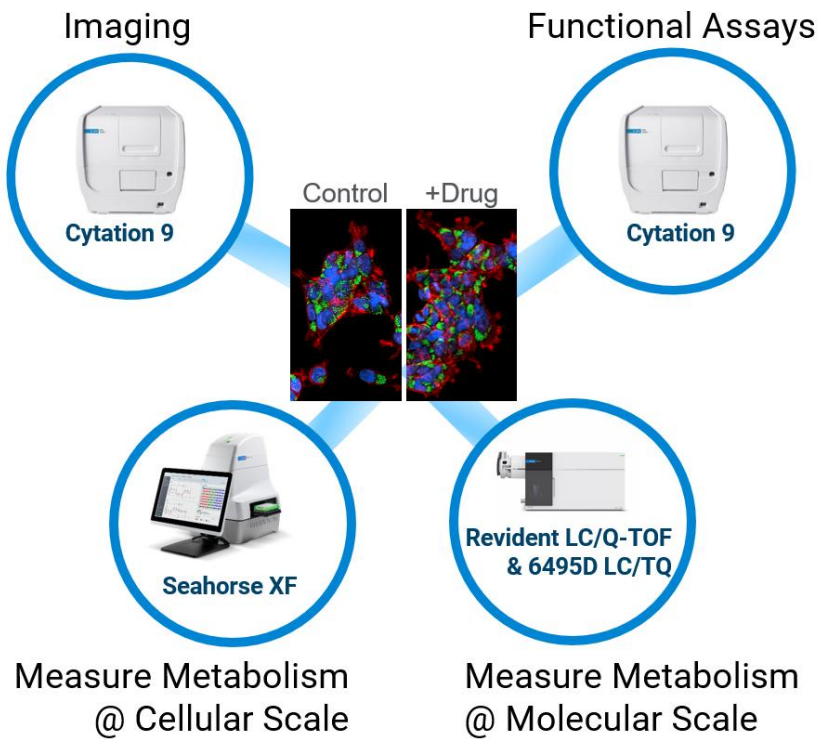


Figure 1. Overview of instrumentation for comprehensive study of cellular metabolism.

Experimental

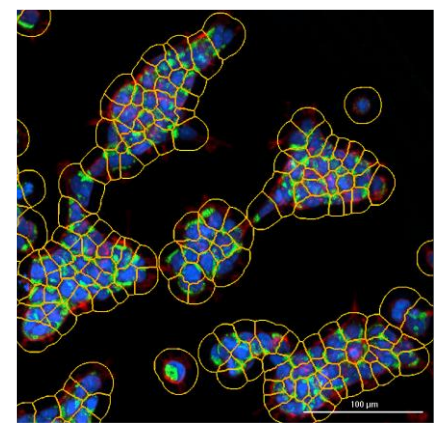
Cell Culture

HEK293 cell line stably expressing GLP1R was cultured in media for 24 hours, after which growth medium was refreshed or replaced with serum free medium, serum free medium (0.1% FBS) supplemented with palmitate-BSA, or various concentrations of palmitic and oleic acid, and/or various concentrations of GLP-1 agonists liraglutide and semaglutide.

Experimental

Imaging and Detection

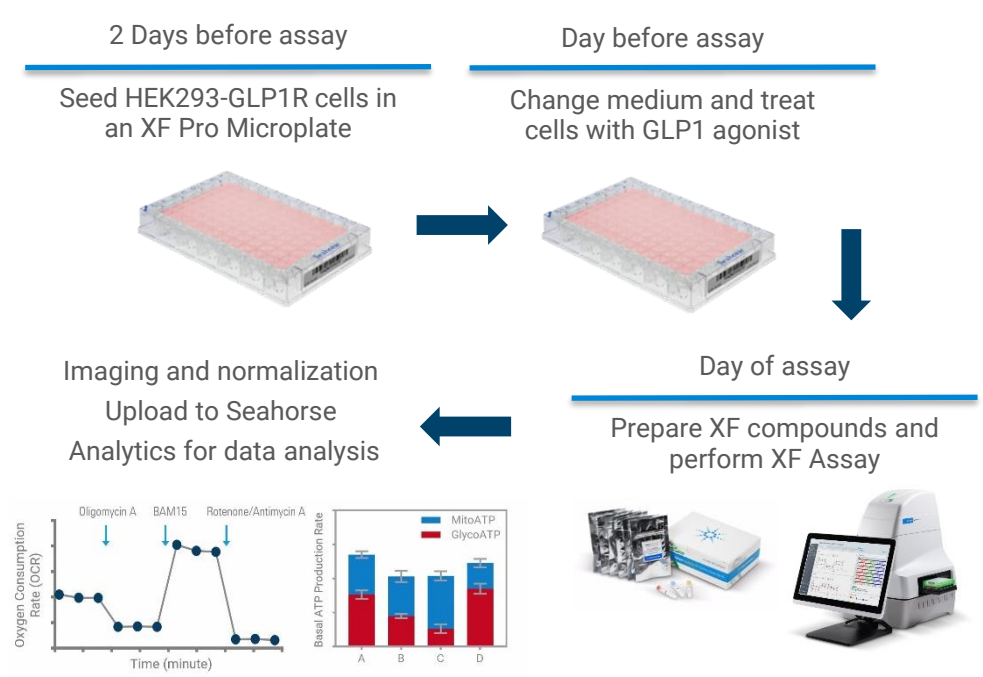
cAMP accumulation assays were performed using an Agilent BioTek Cytation 9 cell imaging microplate reader, which was also used to acquire fluorescence images of cells stained with BODIPY, phalloidin, and Hoechst.



Identify cells → Measure BODIPY Density

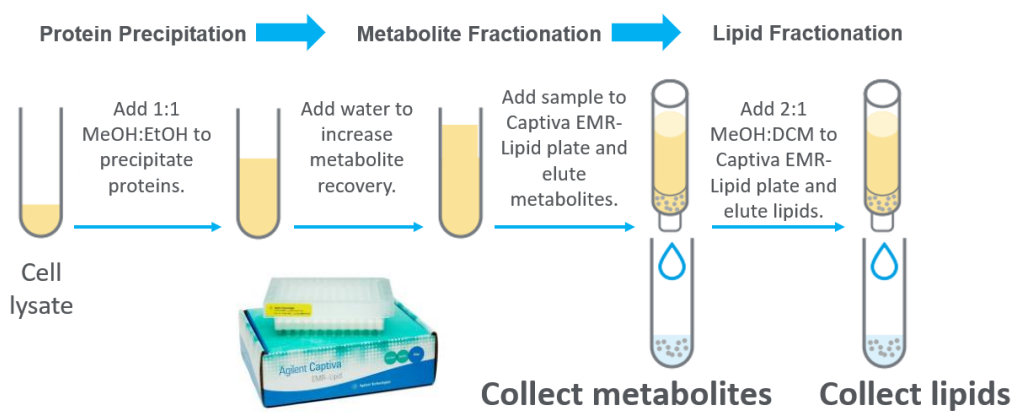
Seahorse

A Seahorse XF Pro analyzer was used to assess cellular bioenergetic phenotypes with the Mito Stress Test.



LC/MS

A manual version of a previously established dual cellular extraction workflow[1] was used. Following room-temp cell lysis and quenching with TFE, metabolite and lipid fractions were prepared:



Metabolite extracts were separated with a standardized HILIC-Z LC method[2] and analyzed in (-) mode with an Agilent 6495D LC/TQ. Lipid extracts were separated with an optimized RP-LC method[3] and analyzed in (+) mode with an Agilent Revident LC/Q-TOF. Data analysis was performed with a combination of MassHunter Explorer 2.0, MassHunter Quant 12.2, LipidMatch Suite 5.73 (Innovative Omics), and Mass Profiler Professional (MPP) 15.1 software.

Results and Discussion

Phenotyping HEK293-GLP1R Cells with Live Cell Imaging and cAMP Assay

To confirm GLP-1R expression a cAMP accumulation assay was performed with Cytation 9. GLP-1R expressing HEK293, but not control HEK293 cells (not shown), responded to application of both agonists at nM concentrations. Live cell imaging with Cytation 9 revealed the total BODIPY intensity per cell increased with FFA concentration, consistent with lipid droplet formation. Similar levels of BODIPY staining were measured w/ and w/o agonist, suggesting GLP1R activation is not altering lipid droplet formation. Interestingly, a morphology difference was noted with agonist treatment.

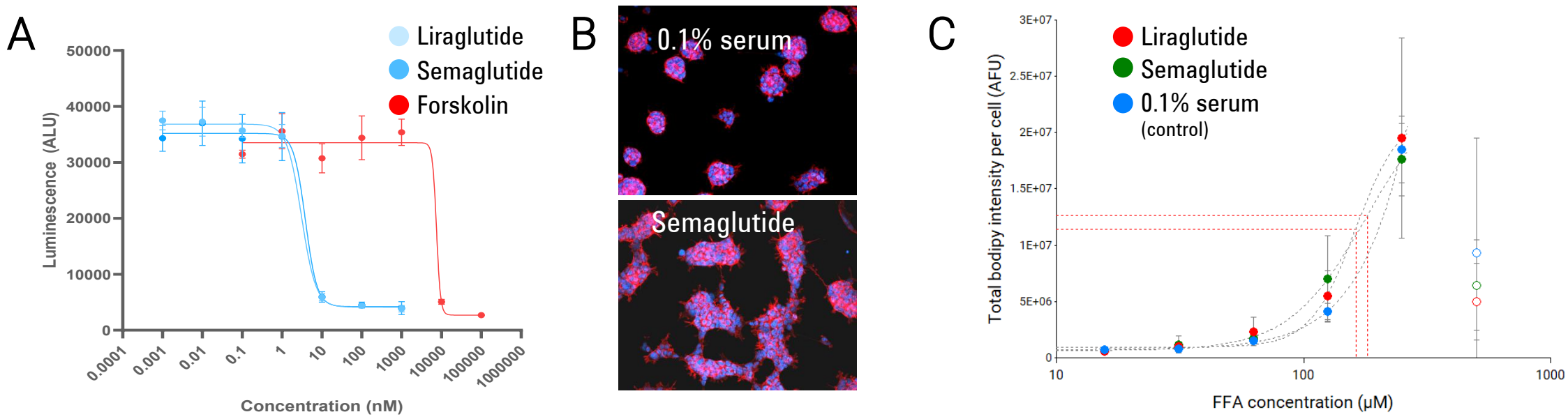


Figure 2. Imaging and cAMP Accumulation Assay Results. (A) cAMP-Glo luminescence assay results. Observed EC50 values for liraglutide (3 nM), semaglutide (4 nM) and forskolin (5 µM, positive control) were similar to previously reported values. (B) GLP-1R agonist-mediated morphology results. (C) HEK293-GLP1R line imaging assay results: FFA uptake +/- GLP1R activation

Measuring Metabolism at Cellular Resolution with Seahorse XF

Seahorse XF experiments revealed that treatment of HEK293-GLP1R cells with liraglutide during 24 hrs resulted in a metabolic reprogramming toward a more glycolytic phenotype independent of media conditions. In addition, when cells were starved of serum for 24 hrs, liraglutide induced a decrease in mitochondrial coupling efficiency and increased proton leak resulting in a significant decrease of mitochondrial ATP production.

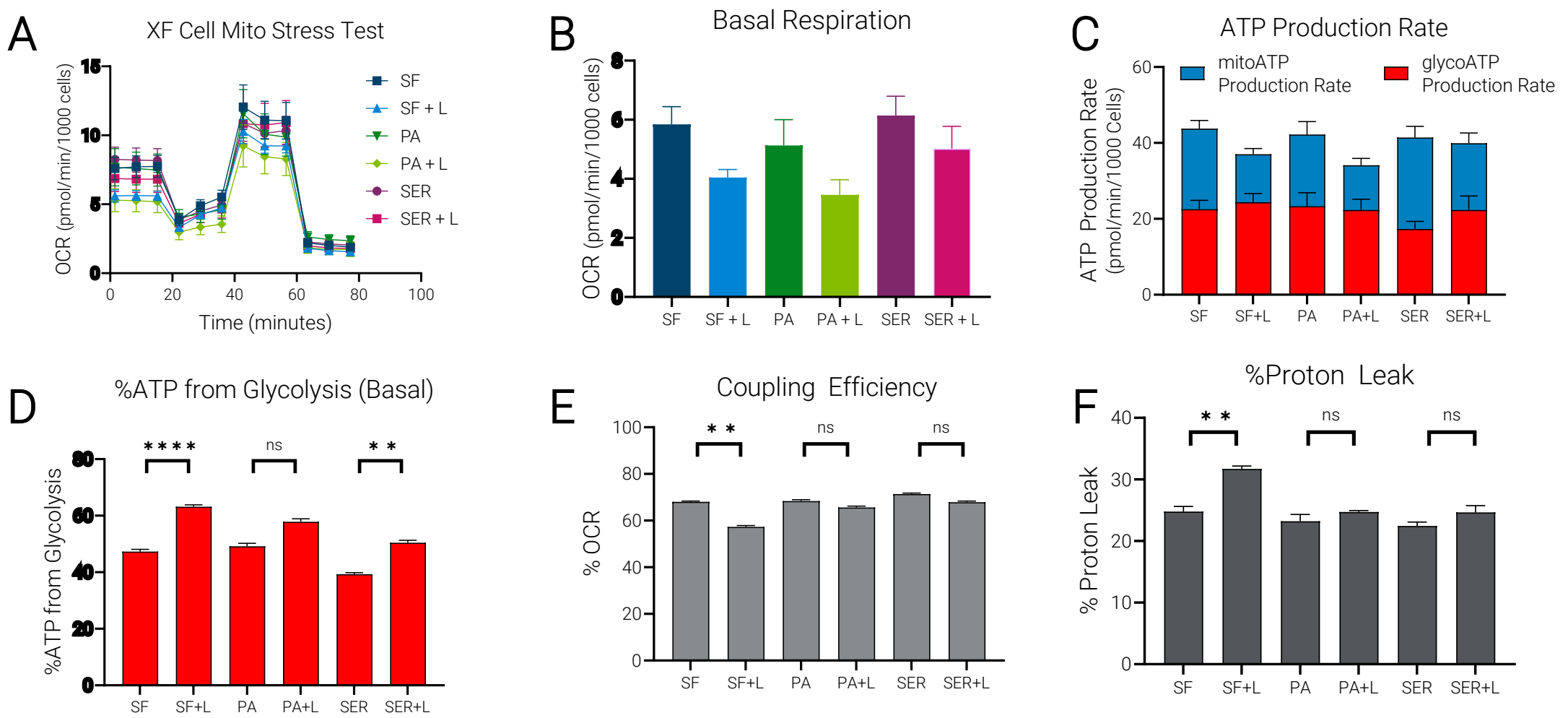


Figure 3. Results of the Cell Mito Stress Test with pretreatment. Representative (A) OCR Kinetic data normalized to cell number, (B) Basal Respiration prior to Oligomycin addition normalized to cell number, (C) ATP Production Rate normalized to cell number, (D) %ATP from glycolysis, (E) Coupling Efficiency, (F) % Proton Leak. Data is presented as Mean ± SEM of 3 independent experiments.
** $p \leq 0.05$ **** $p \leq 0.0001$. SF: Serum Free. PA: Palmitate-BSA. SER: growth medium containing 10% FBS. L: Liraglutide (100 nM).

Results and Discussion

Measuring Metabolism at Molecular Resolution with LC/MS Metabolomics (Mx) and Lipidomics (Lx)

Mx analysis with LC/QQQ detected 222 metabolites. As a first check, an increase in cAMP and a decrease in ATP levels were observed and agreed with the cAMP assay and XF results, respectively.

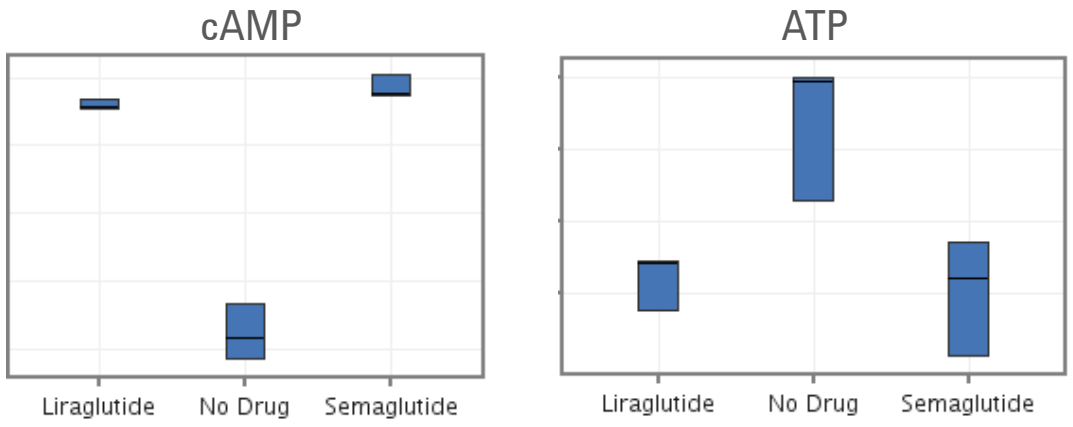


Figure 4. MPP Box-whisker plots of normalized cAMP (left) and ATP (right) abundances measured. Cells fed with FFA O:P 3:1

Differential analysis revealed 71 significant metabolites that were up/down in any of the growth conditions and drug/control pairings.

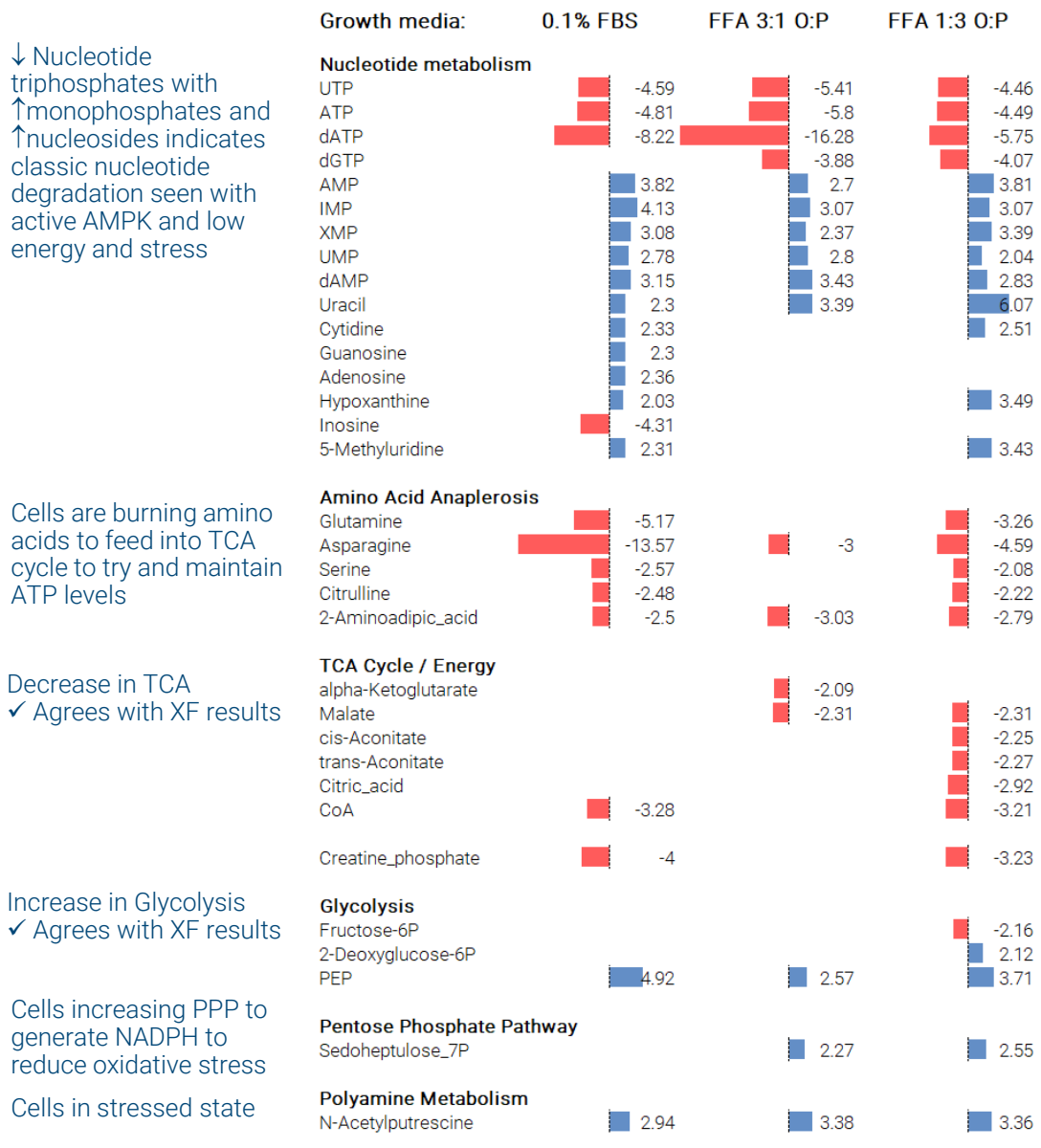


Figure 5. 33 of 71 significant metabolites grouped by pathway with averaged fold-change of liraglutide/semaglutide vs control in t3 growth media conditions. Only values are shown with p-value <0.05. O:P: oleic acid: palmitic acid ratio. Tentative interpretations of pathway changes are in blue.

Lx analysis[4] showed an increase in triacylglycerol content when cells were supplemented with FFA, consistent with lipid droplet formation, and a notable shift in the types of esterified fatty acyls that reflected the supplemented FFA composition. However, GLP1R agonist treatment did not result in significant differences in the lipidome, in agreement with imaging results that lipid droplet formation was not influenced by drug treatment.

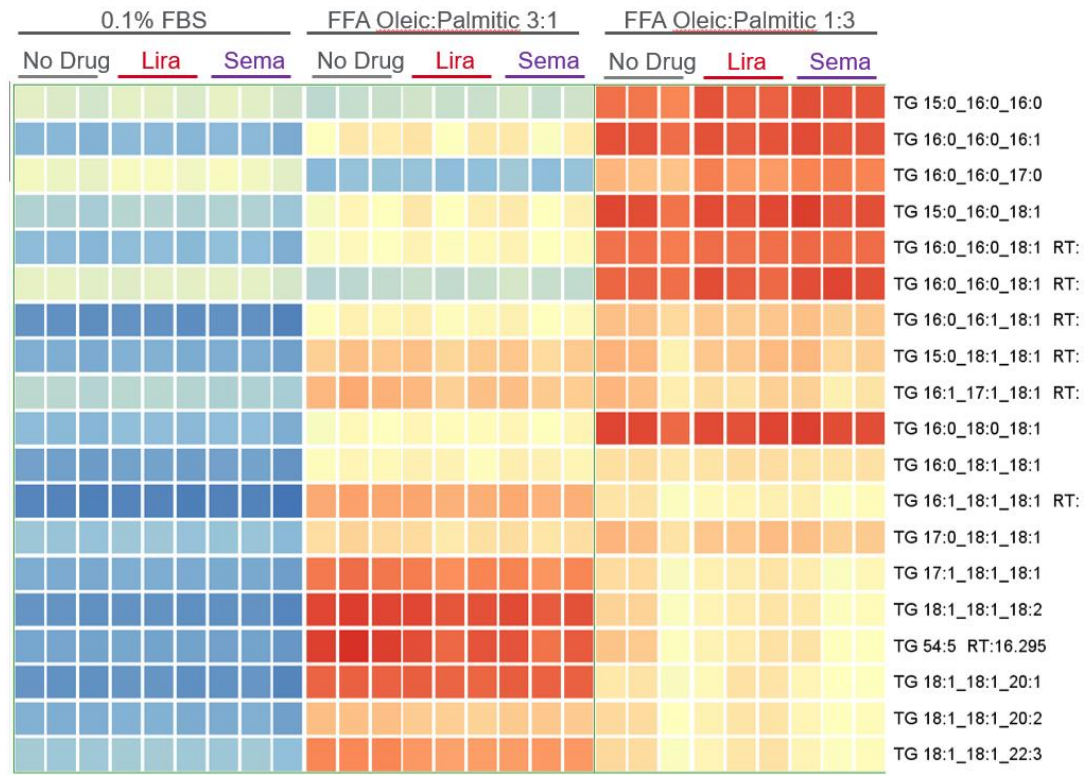


Figure 6. MPP lipid matrix plot of triacylglycerol (TG) abundances across 9 sample groups, 3 reps each, measured by LC/Q-TOF.

Conclusions

Cellular analysis combined with LC-MS provided synergistic insights into GLP1R molecular mechanisms

Under the conditions tested, GLP1R agonist treatment:

- Induced a more glycolytic phenotype
- Caused a decrease in mitochondrial coupling efficiency and increased proton leak
- Did not affect lipid droplet formation

References

[1]Van de Bittner, G et al. An Automated Dual Metabolite + Lipid Sample Preparation Workflow for Mammalian Cell Samples. Agilent Technical Overview 5994-5065EN, 2022.

[2]Yannell, K et al.An End-to-End Targeted Metabolomics Workflow. Agilent Application Note 5994-5628EN, 2023.

[3]Hyunh, K et al. LC/MS dMRM Method Refinement Expands Targeted Lipidomics Studies from Plasma to Cells and Tissues. Agilent Application Note 5994-8365EN, 2026.

[4]Sartain, M et al. Lipid Insight Unblocked: Combining Nontargeted LC/MS Chemometrics With Automated Lipid Annotation. ASMS 2026 Poster WP 504

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Published in USA, June 10, 2026

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