A Quantitative Lipidomic Study for 3D Cell Culture Using Deuterium Oxide Metabolic Labeling

Jonghyun Kim[1], Kyoung-Jin Choi[2], Sung Bum Park[2], Yoon-Ju Na[3], Ki Young Kim[2] and Tae-Young Kim[1]*

[1]School of Earth Sciences & Environmental Engineering, Gwangju Institute of Science and Technology, Gwangju, Republic of Korea,

[2]Therapeutics & Biotechnology Division, Korea Research Institute of Chemical Technology, Daejeon, Republic of Korea, [3]Department of New Drug Discovery and Development, Chungnam National University, Daejeon, Republic of Korea

Abstract

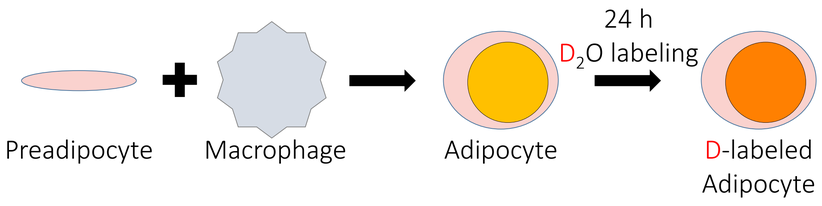
Three-dimensional (3D) cell culture is gaining popularity as a more representative *in vivo* model in contrast to the traditional two-dimensional (2D) cell culture method. Nevertheless, there is no standardized technique for quantifying lipids in 3D cell culture. This study introduces an innovative quantitative lipidomics approach that merges a 3D culture system with deuterium oxide (D_2O) metabolic labeling, offering extensive insights into alterations in metabolism. Preadipocytes and adipocytes were cultured within a hydrogel-based 3D cell, alongside co-cultured macrophage cells that triggered insulin resistance. To comparatively quantify lipids, the technique of D_2O labeling for global omics relative quantification (DOLGOReQ) was employed. DOLGOReQ facilitated the measurement of numerous lipids spanning key categories like glycerolipids, glycerophospholipids, fatty acyls, and sphingolipids. Importantly, the D-labeling efficiency was notably more consistent in 3D-cultured adipocytes in comparison to their 2D counterparts. By utilizing DOLGOReQ, it was determined that the presence of macrophages led to a significant reduction in free fatty acids and long-chain triacylglycerols (TG). The quantitative relationship between TG and free fatty acids indicated that the decrease in TG was a consequence of lowered free fatty acids, which are precursors for lipid synthesis. Furthermore, the macrophages increased D-labeling efficiency, implying an enhancement in lipolysis and subsequent TG reduction. DOLGOReQ not only allows for the assessment of relative quantitative changes but also offers valuable insights into the dynamics of lipid turnover. These findings underscore the potential of DOLGOReQ as an indispensable tool for scrutinizing global alterations in lipid metabolism prompted by external influences in 3D cell culture investigations.

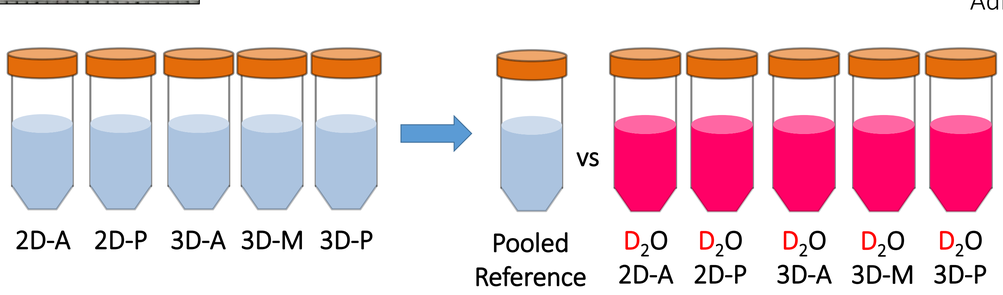
Introduction

- ✓ Cell culture has been the cornerstone of *in vitro* experiments to understand the biological response of cells to different conditions without having to sacrifice animals.
- ✓ However, a conventional 2D cell culture environment on a flat surface is too simple to emulate the complex *in vivo* environment, leading to dissimilar results between cell culture and animal experiments.[1]
- ✓ To bridge the gap, *in vivo*-like 3D cell culture has been developed by imitating cell-cell and cell-environment interactions in tissue structure. To understand physiological differences between 2D and 3D cell cultures at the molecular level, "Deuterium Oxide (D_2O) Labeling for Global Omics Relative Quantification (DOLGOReQ)" was exploited.

Experimental Section

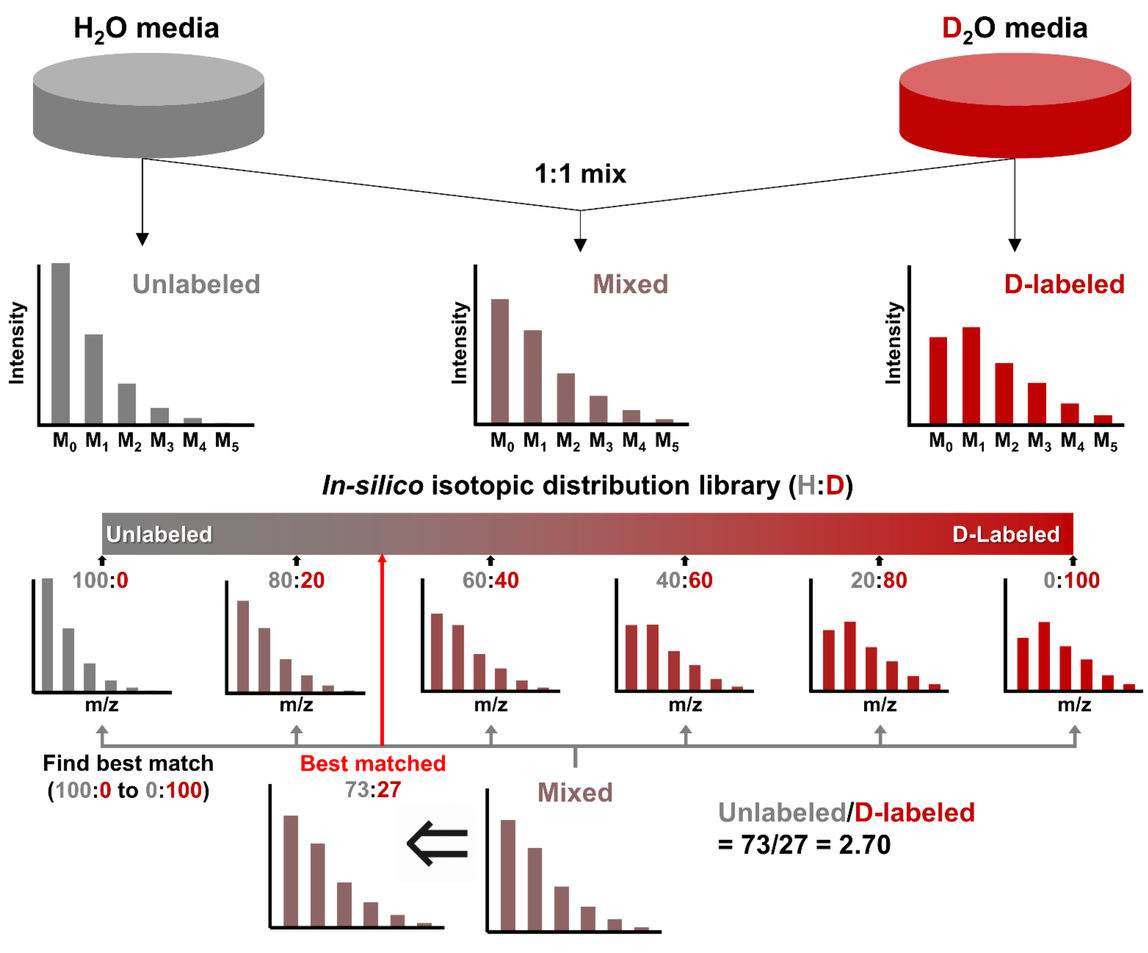
❖ 3D Cell culture & sample preparation





2D/3D: 2D/3D culture A: monocultured adipocyte
P: preadipocyte M: cocultured adipocyte with macrophage

❖ D_2O Labeling for Global Omics Relative Quantification (DOLGOReQ)[2]



Results & Discussion

❖ Determination of a proper macrophage concentration

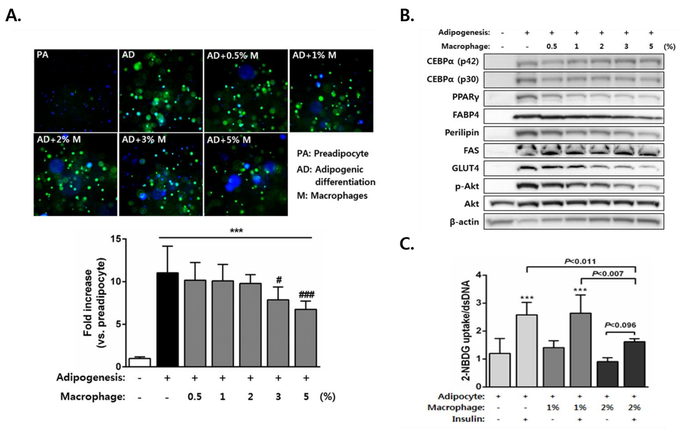


Fig. 1. Optimization of the conditions for macrophage coculture adipocytes. (A) AdipoRed lipid droplet quantitation assay, (B) immuno-blotting, (C) glucose uptake assay.

A concentration of 2% macrophages was found to be the optimal concentration for 3D coculture. This concentration was able to induce insulin resistance without interfering with adipogenic differentiation.

❖ Number of quantified lipid ions

Table 1. Number of quantified lipid ions as a function of the mass isotopic distribution (MID)) measurement and D-labeling

Cell type	Number of lipid ions*	
	MID was measured	MID was measured & D-labeled (Quantified)
2D adipocyte	261±37	222±55
2D preadipocyte	213±47	95±83
3D preadipocyte	209±17	22±15
3D adipocyte	251±36	103±39
3D macrophage cocultured adipocyte	250±2	157±15

*The number of quantified feature is obtained by averaging individual lipid ion features quantified from three biological replicates (mean ± standard deviation).

❖ D-labeling efficiency of adipocytes

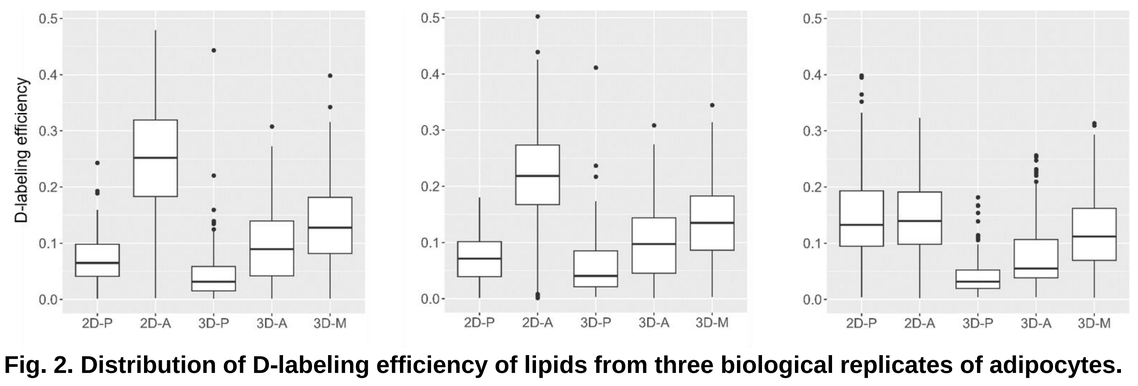


Fig. 2. Distribution of D-labeling efficiency of lipids from three biological replicates of adipocytes.

- ➢ The **2D-culture, adipogenic differentiation, and macrophage coculture** increased D-labeling efficiency and the quantification number of lipids.
- ➢ The relatively low D-labeling observed in 3D cultures is strongly expected to result from the presence of **quiescent** and **hypoxic** cells.
- ➢ From macrophage, **proinflammatory cytokines**[3] are secreted which are involved abnormal lipid metabolism such as **de novo lipogenesis** and **lipolysis.**[4]

❖ Quantification of triacylglycerols (TGs) in adipocytes

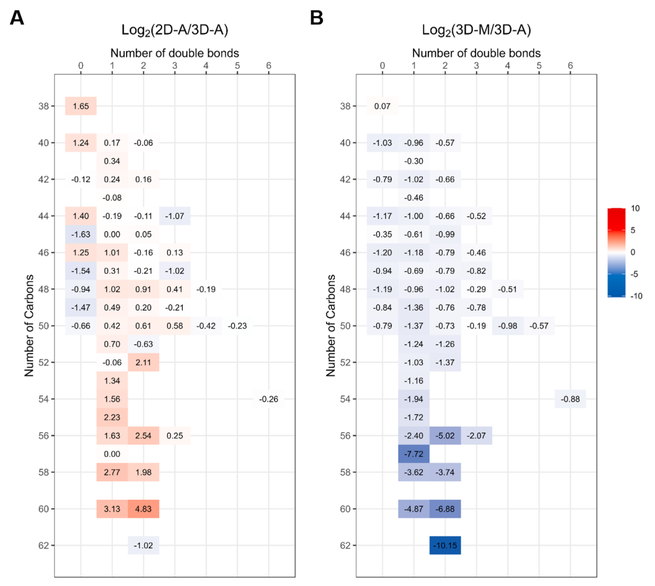


Fig. 3. The relative abundance of TGs in (A) 2D-adipocyte and (B) 3D-macrophage-cocultured adipocytes were compared to 3D-mono-cultured adipocytes. The relative abundance of TGs was presented across the number of carbons and the degree of double-bond unsaturation. The numerical values inside each tile correspond to the average log2-fold change ratio of that lipid species.

- ➢ The amount of TGs in 2D and 3D cultures was not significantly different.
- ➢ However, macrophages reduced the amount of TGs with high carbon numbers in **3D-macrophage-cocultured adipocytes** compared to **3D-monocultured adipocytes**.
- ➢ This is because **long-chain fatty acids** were used as mediators of the **anti-inflammatory response** and were not sufficiently synthesized to compensate.[5]

Conclusions

- ✓ In this study, DOLGOReQ was used to study the lipid metabolism of adipocytes in 2D and 3D cultures. The results showed that 3D coculture with macrophages induced insulin resistance, which could be used as an alternative to animal testing.
- ✓ DOLGOReQ allowed for relative quantification of the **global lipidome** of adipocytes, along with **lipid turnover** from measurements of alterations in mass isotopic distribution due to metabolic D-labeling.
- ✓ The results have demonstrated that **the decrease in TGs with long-chain fatty acyls** during macrophage coculture is a consequences of decreased lipid biosynthesis. This is supported by **a decrease in free fatty acids**, which serves as precursors for the synthesis of TGs.

References

1. Langhans, S. A., *Front. Pharmacol.* 2018, 9, 6.
2. Kim, J. and Seo, S. et al., *Anal. Chim. Acta.* 2023, 1242: 340722
3. Arango Duque, G. et al., *Front. Immunol* 2014, 5, 491.
4. Shi, J. et al., *Front. Endocrinol.* (Lausanne) 2019, 10, 703
5. Shin, S., *J. Obes. Metab. Syndr.* 2022, 31 (2), 147-160

Acknowledgements

We acknowledge support from the National Research Foundation (NRF-2022R1A2C200595512).