Conclusion

Cellular and animal models are imperative in revealing the underlying mechanisms of metabolic diseases and identifying their therapeutic targets. The models aid in bridging the gaps in understanding the metabolic diseases and accelerating the therapeutic development.

This study shows that THP-1 can be successfully differentiated into an ATM subpopulation which closely resembles to those found *in vivo* from obese individuals. This differentiation establishes a cell based model for studying "MMe". Our approach includes simpler methods compared to traditional models offering a reproducible and reliable alternative to relying on isolated macrophages from animal models or human subjects.

Significance of the model:

1. Reproducibility and reliability: Our differentiation protocol ensures consistent results across different experiments and gives reliable results. And this *in vitro* model effectively mimics the characters of macrophages from obese AT. The model recapitulates the cellular and molecular features of ATMs, including their gene expression profiles, cytokine secretion patterns, lipid-handling capabilities, and inflammatory responses.

2. Our approach by-passes the complexities associated with isolating macrophages from animal models or human biopsies. This makes it cost-effective and reduces the reliance on animal models, aligning with ethical standards and bypasses practical difficulties related to it.

3. *Applications*: The model enables researchers to investigate the characteristics and functions of adipose tissue macrophages (ATMs) during obesity. It can aid in uncovering the molecular mechanisms linking chronic inflammation in adipose tissue to systemic insulin resistance and metabolic dysfunction.

It provides a controlled environment to investigate how obesity-associated factors (like free fatty acids, hypoxia, or cytokines) regulate macrophage activation and secretion profiles.

It provides insights into how these macrophages interact with adipocytes, which is crucial for understanding obesity-related inflammation and metabolic dysregulation.

It can be used to explore the potential therapeutic targets for modulating macrophage activity. It serves as a platform for high-throughput screening of anti-inflammatory agents or metabolic regulators.

Limitations:

In vivo vs *In vitro*: It is difficult for an *in vitro* system to fully replicate the aspects of *in vivo* conditions. Enhancing complexity of *in vitro* models to more accurately replicate *in vivo* remains a challenge.

Heterogeneity: The macrophages diversity that does not exist independently in living system. Macrophages are highly heterogeneous (diverse in function and phenotype) depending on their tissue location, environmental signals, and disease conditions. Within each adipose depot, macrophages exhibit spatial variations based on their microenvironment. This becomes a challenge to comprehend their interaction with other cells and adipocytes in the tissues which is important to understand the modulation in their functions during disease condition.

Thus, as an alternative to traditional animal and human models, this cell-based model provides a more ethical, cost-effective, and efficient platform for advancing research related to metabolic disease. By bypassing the complexities associated with isolating macrophages from animal tissues or human biopsies, this model enables researchers to closely examine adipose tissue macrophage (ATM) function and interactions in a controlled environment. Additionally, it simplifies the study of obesity-related inflammation, insulin resistance, and potential therapeutic targets while aligning with ethical research standards by reducing reliance on animal experimentation.

Future Perspective

This study has established an *in vitro* model of MMe, and looked at the key features relevant to the ATMs and obesity, like inflammatory characteristics, surface markers and metabolic traits. This model can be used for deeper understanding of the characters associated with ATMs and obesity.

- Studying the metabolic flux of MMe with and without 2-DG and Etomoxir will give further insights of their metabolic alterations during nutritional stress. Other than this, surface marker expressions (mRNA and proteins) will suggest how modulating metabolism affect their phenotype. M1 and M2 should also be kept as control to find more differential characters of ATMs.
- Dysfunction of autophagy can be verified by using blocker and assessing the levels of LC3. Further, importance of autophagy and lysosomal activity (biogenesis and metabolism) in inflammation and metabolic alterations in MMe (along with M1 and M2) can be investigated.
- Our mass spectrometry data strongly suggests about oxidative stress in these cells. This can be further investigated as to how oxidative stress is linked to inflammation and autophagy especially in context of ATMs. Whether failed autophagy affects the number and quality of mitochondria.
- Mitochondrial number and activity can be investigated among all the subtypes. Looking at the complexes activity can suggest how infection or over-nutritional stress differentially regulate their functions.
- Effect of macrophages secretome on adipocyte metabolism can also explain its functional dysfunction during obesity. Similarly, it can be verified whether MMe can be generated when M0 are exposed to conditioned media of hypertrophic adipocytes.