Chapter 1

Introduction

1.1 Background

Obesity is a major global health concern and a leading contributor to the development and progression of type 2 diabetes mellitus (T2DM). As the ninth leading cause of mortality, diabetes accounts for over 1 million deaths annually (Khan et al., 2019). This burden is further accelerated by its rapid rise. Since 1990, adult obesity has more than doubled, and childhood obesity has quadrupled. As of 2022, approximately 1 in 8 people worldwide was living with obesity (Huang et al., 2025). This growing prevalence is particularly concerning because obesity is strongly associated with insulin resistance, a key pathophysiological driver of T2DM. T2DM is a chronic condition characterised by the body's inability to use insulin effectively, leading to high blood sugar levels (Goyal et al., 2023). Insulin is a hormone secreted by β cells that controls the metabolism of carbohydrates, proteins, and fats by stimulating the uptake of molecules such as glucose from the blood into fat, skeletal muscle, and the liver. A reduction in insulin signaling, primarily in the insulin receptor substrate (IRS)/phosphoinositide-3-kinase (PI3K)/protein kinase B (AKT) axis, triggers insulin resistance that could affect the metabolic actions of insulin (Wondmkun, 2020).

In the context of obesity, these disruptions are closely linked to adipose tissue dysfunction. Obesity is characterised by excessive accumulation and expansion of adipose tissue, not only in classical fat depots but also ectopically in non-adipose organs. This expansion promotes the release of metabolites and pro-inflammatory cytokines, creating a state of chronic low-grade inflammation (Liu et al., 2026). Furthermore, adipocytes themselves are key insulin-responsive cells that play a central role in systemic glucose homeostasis by regulating glucose uptake and lipid storage. Hence, dysfunction in adipocytes directly contributes to impaired insulin signaling and whole-body metabolic imbalance (Imi et al., 2022). Consequently, this inflammatory environment disrupts cellular function,

further impairs insulin signaling, and contributes to metabolic dysregulation. Over time, these changes can lead to β-cell dysfunction, persistent hyperglycemia, and ultimately the progression of T2DM (Ruze et al., 2023). As such, adipose tissue inflammation has emerged as a key mechanistic link between obesity and metabolic diseases, including T2DM.

To investigate the mechanisms underlying adipocyte dysfunction and insulin resistance, reliable in vitro models are needed. The 3T3-L1 pre-adipocyte cell line is one of the most widely used models for studying adipocyte biology, as these pre-adipocytes can be chemically induced to differentiate into mature adipocytes using a standard MDI cocktail containing IBMX, dexamethasone, and insulin (Ahmed & Kim, 2019). While the individual induction of PPARG, FABP4, LPL, ADIPOQ, and GLUT4 during 3T3-L1 differentiation is well documented, no prior study has tracked all five simultaneously across a single time course to establish their relative order of induction. This creates uncertainty, particularly within our own laboratory's use of this model, about which differentiation day is appropriate for downstream functional assays. This study addresses that practical gap by establishing a temporal expression benchmark to guide the selection of differentiation endpoints in future in-house studies of adipocyte dysfunction.

1.2 Objective

The primary aim of this study is to characterise the differentiation of 3T3-L1 preadipocytes into mature adipocytes using morphological and molecular markers (*PPARG, FABP4, LPL, ADIPOQ,* and *GLUT4)*. This aim will be achieved through the following objectives:

- To confirm successful adipocyte differentiation morphologically through the visualisation of intracellular lipid droplet accumulation
- To validate differentiation at the molecular level by assessing the gene expression of key adipogenic markers in differentiated cells using qPCR.

1.3 Hypothesis

It is hypothesised that 3T3-L1 preadipocytes induced to differentiate using the standard MDI protocol will acquire the morphological and molecular characteristics of mature adipocytes by day 14 Specifically, it is predicted that differentiated cells will display visible intracellular lipid droplet accumulation under brightfield microscopy. Additionally, it was hypothesised that adipogenic markers would not be induced uniformly but would follow a staged temporal sequence, with early transcriptional commitment markers (PPARG, FABP4) upregulated prior to markers of metabolic and endocrine maturity (LPL, ADIPOQ, GLUT4). Confirming this sequence would establish a temporal benchmark for identifying differentiation stages in future studies using this model.