Chapter 6

Effect of Macrophages on adipocytes

Expansion of AT leads to impaired function, attributed to factors such as lipotoxicity, ER stress, hypoxia, and metabolic inflammation. These processes are implicated in the progression of IR (Wondmkun, 2020). The turnover of lipids in adipocytes is a critical element in the development of dyslipidemia (Spalding et al., 2008). Consequently, the release of FFAs from adipocytes triggers the UPR through JNK activation, resulting in inflammation and oxidative stress that contribute to the manifestation of insulin resistance in AT. Necrosis of adipocytes induced by hypertrophy represents a significant phagocytic stimulus that governs the infiltration of macrophages into adipose tissue. This infiltration gradually intensifies with the progression of obesity (Cinti et al., 2005), by forming distinctive crown-like structures encircling necrotic adipocytes (Strissel et al., 2007).

Macrophages affect adipogenesis and insulin signalling:

FFAs, crucial adipocyte-derived paracrine mediators, promotes inflammation in macrophages (Suganami et al., 2005). Macrophage-conditioned medium inhibits 3T3-L1 adipocyte differentiation (Constant et al., 2006) and influences insulin sensitivity by downregulating GLUT4 and pAkt transcripts (Xie et al., 2010). Moreover, macrophage-secreted factors modulate mitochondrial activity, impacting the energy homeostasis of human WAT (Keuper et al., 2017). Furthermore, MMe macrophages in a DIO mice, exhibit a dual role, contributing detrimentally by potentiating inflammation and beneficially by exocytosing lysosomes to clear dead adipocytes. This dual function is driven by Nox2 and is dependent on the duration of exposure to a high-fat diet (Coats et al., 2017).

In order to follow the influence of macrophages on adipocytes, we subjected differentiated adipocytes (3T3-L1) to conditioned media from different sub-types of macrophages. Our observations revealed that the conditioned media from both M1 and MMe macrophages exerted diverse effects on the adipocytes. We found the varying effects on adiponectin (AdipoQ) expressions when exposed to conditioned media from both M1 and MMe macrophages. AdipoQ exhibited a non-significant reduction with M1 conditioned media, whereas there was a notable 50% decrease with MMe conditioned media. In contrast, M2 conditioned media did not induce any alterations in AdipoQ levels (Fig 6.1 A). This reduction in adiponectin secretion serves as a significant marker of insulin resistance. Adiponectin has anti-inflammatory effects, helping to mitigate inflammation in various tissues, including adipose tissue and the vascular system (Ouchi et al., 2003). It enhances insulin receptor substrate (IRS) phosphorylation and Akt activation, facilitating downstream

insulin actions (Kadowaki, 2006). Furthermore, Adiponectin is involved in the regulation of mitochondrial biogenesis and enhanced mitochondrial function is associated with insulin sensitivity (Guo et al., 2020).

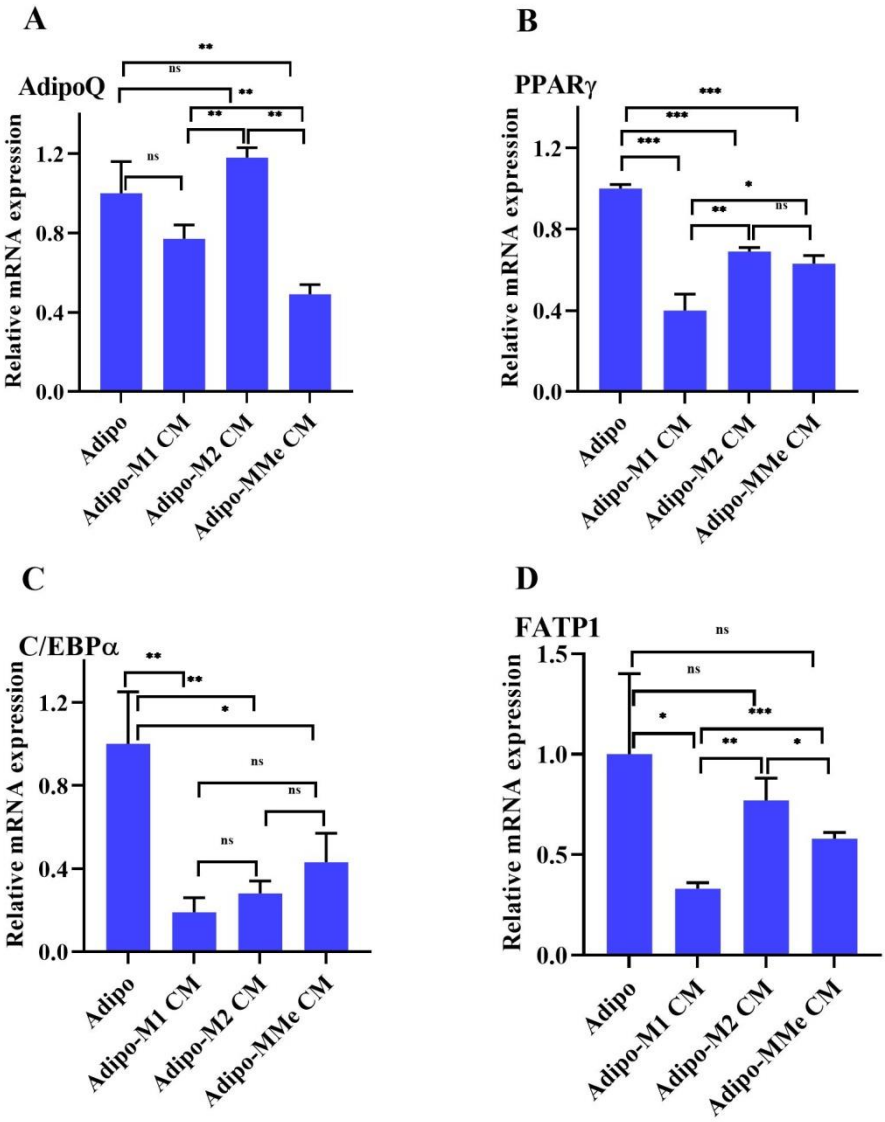

Figure 6.1 Effect of macrophage-conditioned media on the adipocyte: (A-D) Differentiated 3T3-L1 adipocytes were subjected to conditioned media (CM) from M1, M2, and MMe macrophages. Relative mRNA expression of (A) AdipoQ, (B) PPARγ, (C) C/EBPα, and (D) FATP1 was analyzed. Data represented Mean±SD (n=3), *p<0.05, **p<0.01, ***p<0.001, ns= not significant**.**

Intriguingly, the conditioned media from MMe did not much affected the adipogenesis markers PPARγ and C/EBPα, unlike the noticeable impact observed with M1 conditioned media (Fig 6.1 B and C). On the other hand, M2 CM maintained PPARγ and C/EBPα levels that support adipogenic process (Fig 6.1 B and C). PPARγ and C/EBPα are key transcription factors that is important for adipogenesis, the process of differentiation and maturation of preadipocytes into mature adipocytes. PPARγ is considered a master regulator of adipogenesis (Rosen et al., 1999). It orchestrates the expression of genes involved in lipid metabolism, adipocyte differentiation, and insulin sensitivity. C/EBPα acts synergistically with PPARγ to drive the terminal differentiation of adipocytes (Ma et al., 2018). It regulates the expression of various adipocyte-specific genes and promotes the formation of mature adipocytes (Rosen et al., 2002).

However, MMe conditioned media lead to a reduction in FATP1, albeit to a lesser extent compared to the effect seen with M1 conditioned media (Fig 6.1 D). FATP1 is a protein that plays a role in fatty acid metabolism. FATP1 has been identified as a regulator of adipogenesis. It contributes to lipid metabolism by facilitating the uptake of fatty acids, a critical step in the synthesis of triglycerides during adipogenesis (Huang et al., 2021). MMe CM affected the lipid uptake which is linked to IR. Altered expression of FATP1 has been correlated with insulin resistance (Wu et al., 2006). On the other hand, PPARγ plays a significant role in insulin sensitivity by influencing adipose tissue development and function.

Changes in AdipoQ indicate that while M1 might not be a significant factor in insulin resistance development however, it does prevent further lipid accumulation in the adipocytes, as seen by PPARγ and C/EBPα levels. The levels of FATP1 are also indicative of the same trend.

To further verify this, we examined the levels of pAkt. It was noted that both M1 and MMe conditioned media led to a reduction in pAkt levels, while the impact was less pronounced with M2 (Fig 6.2 A, B). It became discernible that M2 exhibited a superior ability to maintain insulin sensitivity compared to M1 and MMe. This indicates the dynamic role of MMe in regulating adipocyte homeostasis.

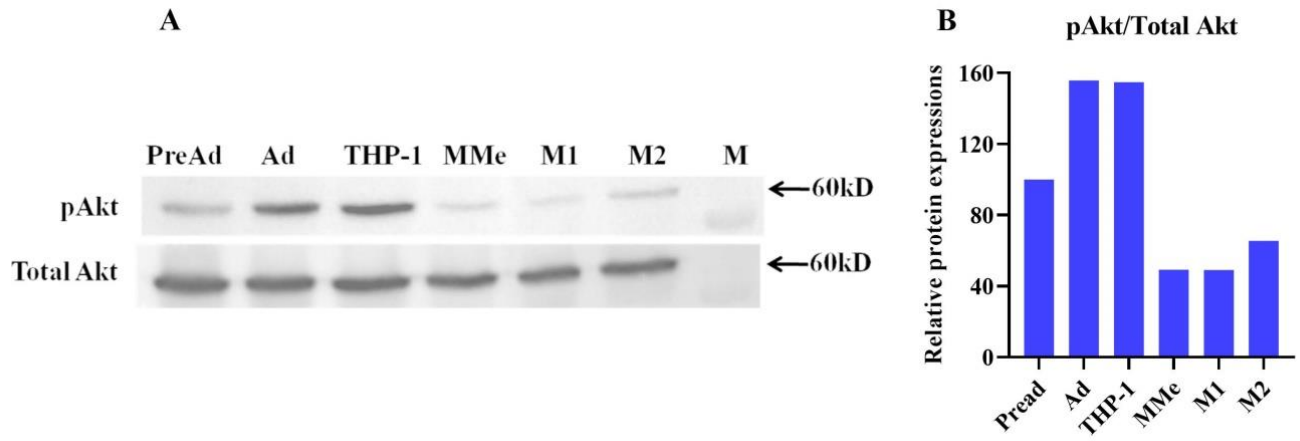

Figure 6.2 Influence of macrophage-conditioned media on Akt phosphorylation in 3T3-L1 adipocytes: (A) Representative immunoblot analysis of phosphorylated Akt (pAkt) and total Akt in preadipocytes (PreAd), differentiated adipocytes (Ad), and adipocytes treated with conditioned media from THP-1, MMe, M1, and M2 macrophages. Protein bands correspond to the expected molecular weight of 60 kDa. (B) Quantification of pAkt/Total Akt protein levels, normalized to untreated adipocytes (PreAd). (n=2)

Discussion:

The expression of Monocyte Chemoattractant Protein-1 (MCP-1) is significantly up-regulated in WAT in the context of HFD-induced obese mice. The elevated MCP-1 expression is associated with deleterious effects on insulin-stimulated glucose uptake and the expression of key regulatory molecules involved in lipid metabolism (Takahashi et al., 2003). The secretion of MCP-1 from adipocytes serves as a direct trigger for the recruitment of macrophages to adipose tissue. This causes local inflammation and ultimately leads to systemic IR (Kanda, 2006). Adiponectin expression, on the other hand, may be indirectly influenced through interactions between adipocytes and MCP-1-expressing ATMs, contributing to alterations in obesity-induced inflammatory genes expressions (Weisberg et al., 2006).

While adipocytes play a central role in orchestrating local microenvironment changes, resident macrophages exhibit notable heterogeneity in their activities and functions (Gordon

& Taylor, 2005). Diet-induced obesity disrupts tissue homeostasis, leading to type 1 inflammatory responses in visceral adipose tissue (Wensveen et al., 2015). PPARγ emerges as a critical signalling molecule in defining the macrophage phenotype in adipose tissue (Charo, 2007), and the reduced PPARγ protein level is linked to increased infiltration of macrophages in the visceral adipose tissue of obese individuals. Macrophage-secreted factors impede insulin signalling in adipocytes by downregulating GLUT4 and insulin receptor substrate-1 (IRS-1). This cascade results in decreased Akt phosphorylation and impedes insulin-stimulated GLUT4 translocation to the plasma membrane (Lumeng, DeYoung, & Saltiel, 2007).

As mentioned earlier, MMe in mice demonstrates a dual role with both detrimental and beneficial functions (Coats et al., 2017). Additionally, our observations revealed that while MMe has a comparatively lesser impact on adipogenesis, it downregulates adiponectin expression, possibly attributed to cytokine secretion by macrophages. This highlights the complex interplay between adipocytes and macrophages and their collective role in the development of obesity-related metabolic dysregulation.

Our experimental results, although not comprehensive, point to the fact that MMe may be bigger driver of insulin resistance. On the other hand, M1 prevent recruitment of new adipocytes. Based on these findings we are tempted to predict that M1 macrophages might be a dominant population in lean diabetics while MMe might predominate in obese diabetics.