The Impact of Oleic Acid on Developmental Phases of Adipocytes and its Contribution to the Development of Obesity-Related Metabolic Disorders

LAY ABSTRACT

Adipose tissue is the primary storage unit for fat in the body and performs significant roles in hormone release. Excessive fat consumption disrupts complex and balanced metabolic mechanisms. Oleic acid, found in olive oil, has previously performed tested benefits in cardiovascular health, though oleic acid has identified to have adverse effects on cellular properties of adipocyte cells, of which adipose tissue is comprised of. It can cause significant changes in cell shape, size and hormone secretion patterns, granting attention for further experiment and analysis. Where adipose tissue is comprised of adipocyte cells, adipocytes are made from pre-adipocytes. There is limited understanding of the effects that oleic acid has on the development of pre-adipocytes into mature adipocytes. In this project we hypothesize that consumption of oleic acid changes the development of a pre-adipocyte and that it further affects behavioural outcomes of a mature adipocyte. This study will focus on the impact of oleic acid on developmental phases of adipocytes *in vitro*, using a 3T3-L1 pre-adipocyte model.

Cell culture and ELISA kits will be used to observe cellular properties and secretions of adipocytes during developmental stages, to identify the impacts of oleic acid on the maturation process and subsequent behaviours, which may encourage development of metabolic disorders, such as *diabetes mellitus* and cancers. Expectantly, cells treated with higher concentrations of oleic acid will cause changes in morphology and secretion patterns, compared to control cells. Data collected from this project may illustrate greatly beneficial understanding in the how dietary consumption of fat interacts with the development of pre-adipocytes, contributing to the complex ways that obesity can develop.

1.0 INTRODUCTION

Despite growing understanding of the scientific background of obesity, the population affected continues to rise. Research has shown the implications of excessive fat accumulation (*Agha & Agha 2017*) leading to hypertrophy and hyperplasia of mature adipocytes (*Jo et al, 2009*), and the effects that dietary fats such as oleic, palmitic and stearic acids have on our health (*Manilla-Mora, 2021*). Some studies suggest that increased concentrations of oleic acid (OA), significantly more so than other dietary fats, have adverse effects on mature adipocytes (*Malodobra-Mazur et al, 2019*). OA was observed to cause morphological changes, impact adipokine secretion, promote adipogenesis and impair insulin signalling. This study will focus on the impact of OA on developmental phases of adipocytes *in vitro*, using a 3T3-L1 pre-adipocyte model. Observing the morphology and cellular properties of developing treated pre-adipocytes may aid in identifying the impact OA has during the maturation process and the subsequent behaviours that could lead to an onset of metabolic disorders, such as diabetes, cancers and vascular diseases.

1.1 OLEIC ACID

OA is a monounsaturated 18-carbon fatty acid, with one *cis*-double bond situated at 9-carbon position from the methyl end. Commonly referred to as *omega* 9, it is the most broadly distributed natural fatty acid (*Choi& Rhee, 2010*). Though obtained from the diet, OA is endogenously synthesised in the human body from essential fatty acids (*Farag & Gad, 2022*). Many studies discuss the benefits of OA in the body, such as reducing cardiovascular diseases, anti-inflammatory and anti-cancer actions (*Rocha et al, 2020*) . Nevertheless, surplus consumption of fatty acids contributes to obesity and obesity-related metabolic disorders (ORMD), with excessive OA concentrations displaying a wide range of significant adverse effects on homeostatic conditions (*Malodobra-Mazur, 2019*).

1.2 OBESITY AND OBESITY-RELATED METABOLIC DISORDERS

Obesity is characterised by excessive storage of fat, abnormal body fat accumulation, exceedance of a body mass index (BMI) of 30 and is considered a key factor in the development of metabolic diseases (*Manna & Jain, 2015*). Adipose tissue (AT), comprised of mature adipocytes, impacts metabolism via secretion of hormones, and other such substances (*Fonseca-Alaniz et al, 2007*). With obesity, the secretion of these substances is increased, thus distressing normal metabolic mechanisms. Their involvement in the development of insulin resistance (IR) and impairment of pancreatic β-cells leads to the onset of DM (*Al-Goblan et al, 2014*). Increased levels of blood glucose, insulin and insulin-like growth factor-1 (IGF-1) are associated with increased risk of developing colon, kidney, and prostate cancers (*NCI, 2022*). Moreover, obesity causes total blood volume and cardiac output to increase, thus elevating cardiac workload significantly, leading to vascular diseases such as myocardial infarction (*Manna & Jain, 2015*). In hypothesising how OA concentrations impacts the outcome of treated pre-adipocytes, we may further understand how dietary consumptions of OA impacts the behaviour of affected adipocytes and subsequent contribution to development of ORMD.

2.0 LITERATURE REVIEW

2.1 OBESITY EPIDEMIC

Obesity was first recognised by the World Health Association (WHO) in 1948. It was disregarded as a metabolic disease and potential threat to human health in the field of medical professionalism until 1997, when the drastic effects of excessive fat accumulation became abundantly clear (*James, 2009*). The NHS Health Survey for England presented data to show that obesity inclined sharply between 1993 and 2000, followed by a consistent, yet annual increase (*NHS, 2020*). Subsequently in 2019, it was recorded that 29% of adult women and 27% of adult men were obese (*Agha & Agha, 2017*). The National Child Measurement Programme (NCMP) recorded a noticeable prevalence of obesity in children, escalating from 20.2% in 2018/19 to 25.5% in 2020-21 (*NCMP, 2022*). Obesity is a chronic disease that has multifactorial origins (*Grundy, 1998*), thus creating a complex network of issues amongst scientific understanding in this field.

2.2 ADIPOCYTES AND ADIPOGENESIS

Through evolutionary progression, adipocytes reserve an impressive ability to expand and store energy, secrete hormones and in turn, hold a strong influence on feeding behaviour and metabolic homeostasis. Adipocytes are adapted to hypertrophic abilities, precisely, to increase cell size to safely store accumulation of intracellular TAGs, to avoid lipids circulating the blood plasma. Nevertheless, adipocytes have a limited capacity for safe storage of TAGs, where exceedance of this limit results in hyperplasia, where cells proliferate to accommodate the additional lipids (*Jo et al, 2009*). Mesenchymal stromal cells, pre-adipocytes differentiate and form into adipocytes via adipogenesis with an ability for self-renewal and multipotential differentiation (*Miana & Gonzalez, 2018*). Thus, adipocytes have various developmental origins, suggesting that the fate of the development of a mature adipocyte likely depends on both intrinsic and extrinsic factors, i.e., lifestyle, heritage and diet (*Miana & Gonzalez, 2018*).

A study conducted an experiment on two mouse strains where each obesity-resistant FVB/N and obesity-prone C57BL/6 strain were fed a high-fat diet (*Jo et al, 2009).* The study concluded that the hypertrophy of adipocytes strongly correlated with diet in both strains. However, hyperplasia appeared to be dependent on genetics, suggesting a synergistic relationship between environment and diet, and genetics. Another study discusses the differences noted between developmental and adult adipogenesis, where hypertrophic adipocyte growth in the adult, highly correlated with development of metabolic disorders (*Ying & Simmons, 2021*). Contrastingly, the development of neonatal adipocytes was observed, where the growth of adipocytes was hyperplastic. The study suggested that hypertrophic growth, particularly, abdominal visceral WAT, and mesenteric WAT (WAT between organs), most correlated with metabolic disorders (*Ying & Simmons, 2021*). Identifying the effects of treating developing pre-adipocytes may prove largely beneficial in observing the behavioural traits of environmentally affected adipocytes, and the speed at which, compared to control cells, hypertrophic and hyperplastic cell growth occurs, thus contributing to increasing greater AT mass.

2.3 PRE-ADIPOCYTES

There are growing bodies of evidence that suggest the impact of fatty acids on mature adipocytes and the lending predisposition of ORMD (*Arimochi et al, 2016*). Damaged or affected pre-adipocytes, however, have not been so widely acknowledged. Pre-adipocytes comprise a substantial proportion of the cells in AT. Though primarily fat cell precursors, they produce hormones and metabolic signals nonetheless, distinct from mature adipocytes (*Florido et al, 2010*). They have important immunological, proinflammatory and haemostatic roles.

Pre-adipocytes should not be discounted as a major influence on the onset of obesity, as the potential dysfunction of a pre-adipocyte could prove to have negligent and adverse effects as mature adipocytes. Adipocytes do not divide mitotically, indeed they recruit locally resident pre-adipocytes which subsequently differentiate into mature adipocytes, in the presence of growth factors secreted by adipocytes during adipogenesis (*Xu et al, 2017*). The signalling functions of some released bioactive substances and adipokines, enhance the differentiation of pre-adipocytes.

In figure 1, we can identify the phases involved in developmental processes (*Peterson et al, 2016*).

Figure 1.

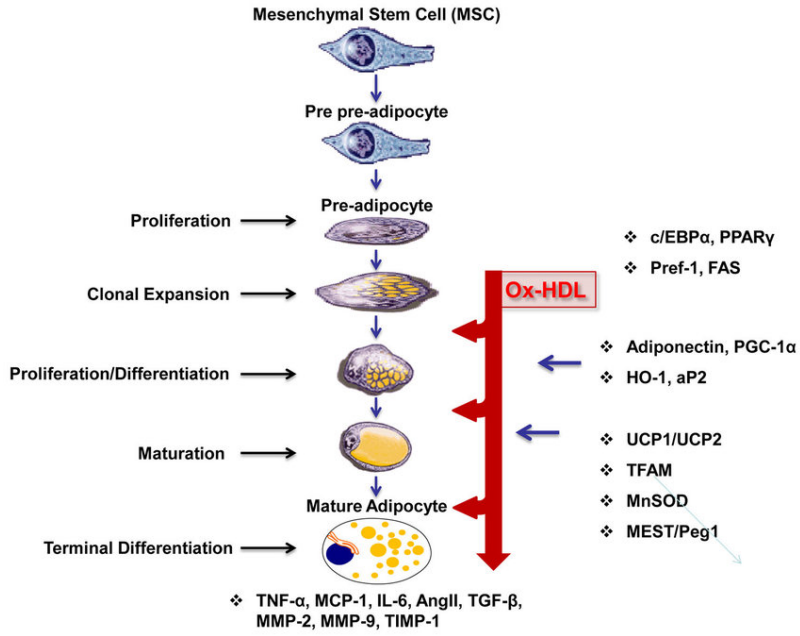

Figure 1 shows the developmental phases of a pre-adipocyte into a mature adipocyte in adipogenesis (*Peterson et al, 2016*).

As seen in figure 1, the various phases of development and precise concoctions of transcription and growth factors involved, such as c/EBPα, PPARγ, adiponectin and IL-6, are considered to play key roles in the fate of behavioural properties in mature adipocytes (*Lai et al, 2012*). A change in secretion patterns and expression rates could dramatically alter the phenotype of an affected mature adipocyte. A study conducted findings that, adipocytes, analysed from obese participants, stimulated proliferation of pre-adipocytes significantly more so than in participants with classified normal BMI (*Lai et al, 2012*). The data resulted in postulation that the expansion of AT in obesity, occurs principally through hyperplastic cell growth, contrasting the findings of work done by Ying and Simmons. Ultimately, the accumulation of adipocytes, whether through hypertrophic or hyperplastic growth, contributes, nevertheless to the complexities of developing ORMD.

2.4 ENDOCRINE FUNCTIONS OF ADIPOSE TISSUE

Collectively, AT is considered a major endocrine organ, having profound influences on the metabolism of body tissues, immunological responses, insulin sensitivity and cardiovascular disease (*Fonseco-Alaniz et al, 2007*). When in health, the body functions in equilibrium and metabolic mechanisms synchronise. Studies have shown that the functions of WAT go far beyond the storage of TAGs in adipocytes, it further plays a significant endocrinal role in production of hormones and bioactive substances such as adipokines (*van Beek et al, 2015*). Adipokines, namely Interleukin-6 (IL-6), contribute to the regulation of food intake, directly impacting weight gain (*Glund & Krook, 2008*). IL-6, primarily, mediates acute phase responses to inflammation; through persistent activity as a proinflammatory cytokine, however,

this develops into chronic inflammation (*Izquierdo et al, 2019*). One study discusses the possibility that with increased body fat content, IL-6 plasma levels are elevated thus implicating a proinflammatory state, involved in the progression of IR, a metabolic disorder associated with obesity (*Hoene & Weigert, 2008*).

Body fat distribution performs a significant part in assessing the risk of disease in obese individuals (*van Beek et al, 2015*). Deposited around the abdomen and surrounding internal organs, visceral WAT is shown to secrete up to three times the amount of IL-6 than in WAT distributed subcutaneously(*Izquierdo et al, 2019*). In obesity, excessive fat consumption causes stress to adipocytes, further causing them to increase their release of FFAs and pro-inflammatory adipokines. These signal the induction of cell infiltration and dysfunctions associated with obese individuals. The pathophysiological differences in WAT, distributed in various areas of the body, are linked to onset of ORMD. It is noted that in comparison to subcutaneous WAT, visceral WAT releases significantly more FFAs and bioactive substances that disrupt harmonious functions of lipid metabolism (*van Beek et al, 2015*).

2.5 LEPTIN SECRETION FROM ADIPOSE TISSUE

AT is also identified to secrete leptin, a substance involved in stimulating dopamine uptake, thus fulfilling satiety (*Volkow et al, 2011*), and inhibits secretion of insulin (*Fernandez & Sanchez, 2013*). Leptin is intrinsically involved in the regulation of energy balance and is associated with expression of neuropeptide Y (NYP) and agouti peptide (AgRP), where both are inhibited and involved in increasing nutritional absorption and decreasing energy consumption (*Baver et al, 2014*). In other regions, leptin stimulates the expression of neuropeptides: proopiomelanocortin (POMC) and cocaine-and amphetamine-related transcript (CART), inducing the inhibition of nutritional intake and promoting energy consumption (*Lartigue et al, 2007*). A study focused on how OA influences adipogenesis of 3T3-L1 pre-adipocytes and how they may predispose obesity-related disorders, examines the expression rate of the leptin gene (*Malodobra-Mazur et al, 2019*). The adipocytes treated at both 0.25mM and 0.5mM concentrations of OA expressed leptin secretion more than five times compared to control cells.

Paradoxically, in terms of lipid metabolism, leptin essentially reduces food intake and increases energy expenditure. Though in obese individuals, with high levels of circulating leptin, they appear to have leptin-resistance and ultimately, become insensitive to the functions of leptin (*Izquierdo et al, 2019*). Leptin is also involved in stimulation or inhibition of cell growth, where high levels of leptin can promote abnormal cell proliferation, thus exposing the development of cancerous cells. Recent records show that 1 in 20 cancers are caused by excess weight (*NCI, 2022*). AT secretion is identified as a key contributor to the pathogenesis of obesity, through an interruption of increased secreted substances that disturb normal energy metabolism. The hormones and substances secreted from adipocytes in AT can be identified in table 1 and is noted that imbalances in these substances will subsequently have disturbed effects on energy utilisation, metabolism, and homeostasis, further contributing to ORMD (*Fonseca-Alaniz et al, 2007*).

Table 1. Hormones and Substances Secreted from Endocrinal Adipose Tissue

Substance	Biological effect
Leptin	Signals to the CNS about the body's energy stocks
Adiponectin	Increases sensitivity to insulin, is antiinflammatory and attenuates the progression of atherosclerosis
Resistin	Increases insulin resistance
TNF-α	Lipolytic, increases energy consumption and reduces sensitivity to insulin
Interleukin-6	Proinflammatory, lipolytic, reduces sensitivity to insulin
Adipsin	Activates the alternative complement pathway
ASP	Stimulates triacylglycerol synthesis in WAT
Angiotensinogen	Precursor of angiotensin II, involved in regulating arterial blood pressure
PAI-1	Inhibits plasminogen activation, blocking fibrinolysis
Tissue factor	Initiates the coagulation cascade
VEGF	Stimulates vascular proliferation (angiogenesis) in WAT
Visfatin	Insulinomimetic predominantly produced by visceral fat
Monobutyrin*	Vasodilator and inducer of vascular neoformation
TGF-β	Regulates a series of processes in WAT, including proliferation of preadipocytes and differentiation, development and apoptosis of adipocytes
IGF-1	Stimulates proliferation and differentiation of adipocytes
HGF	Stimulates differentiation and development of adipocytes

Table 1 shows several hormones and substances that have been identified to be secreted from endocrinal AT. It displays a brief description of their roles and impacts on the body, lending to how imbalances may lead to development of ORMD (*Fonseca-Alaniz et al, 2007*).

2.6 OLEIC ACID ON METABOLIC STATE OF ADIPOCYTES

Adipocytes are the primary location for energy storage that comprises AT, where triglycerides (TAGs) are stored for later utilisation (*Hames et al, 2015*). White adipose tissue (WAT) stores single lipid droplets and plays an influential endocrine role in the human body (*Ying & Simmons, 2021*). Obesity is associated with increased levels of circulating free fatty acids (FFAs), that reflect adipose tissue mass (*Nascimento et al, 2009*). A rise in circulating FFAs can trigger incomplete oxidation of fatty acids, therefore producing reactive oxygen species correlated with mitochondrial stress, potentially leading to cell damage and insulin resistance (*Manna & Jain, 2015*). An FFA that is commonly identified in circulating blood plasma and is consumed in the diet is, OA.

In a study performed by Mantilla-Mora et al, the effects of palmitic acid and OA are addressed on cell response to insulin and simulation of obese phenotypes (*Mantilla-Mora et al, 2021*). In this study, it is identified that concentrations of 500uM of palmitic acid or OA decreased cell viability, thus adipocytes were treated with concentrations of 250-500uM. The result of this study concluded that adipocytes treated with palmitic acid and OA caused alterations to cell secretions of growth factors and patterns of cytokines, as a response to the metabolic changes of lipid overload. Cell diameter was measured after treatment with 500uM of OA, where cell hypertrophy increased significantly more so than that of 500uM palmitic acid. This body of evidence may postulate that, not only does OA alter the pattern of cytokines and other such substances secreted but appears to further encourage and stimulate the storage of TAGs.

HYPOTHESIS 3.0

The hypothesis for this project is consumption of oleic acid impacts the development of a pre-adipocyte and it further affects behavioural outcomes of a mature adipocyte. It is anticipated that treated pre-adipocytes would differentiate into mature adipocytes that secrete increased levels of leptin and IL-6. In doing so, the data obtained can highlight the potentially increased likelihood of developing ORMD such as type 2 *diabetes mellitus* (T2DM) and various cancers.

It is considered that the nature of hypertrophic cell growth was dependent on diet, whereas hyperplastic cell growth is believed to be more dependent on genetics. Proceeding this project, the experiments performed will expectantly provide results that indicate the effects of OA concentrations on the speed at which matured adipocytes, differentiated from treated pre-adipocytes, grow and expand. Based on the research carried out, it would be expected that cells treated with higher concentrations of OA would proceed to display morphological changes and an alteration in secretion patterns, compared to that of control cells. The data collected from this project may illustrate a beneficial understanding in the many complex ways that obesity can develop and how it continues to prevail as a global epidemic.

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