Recent decades have seen a tremendous increase in obesity all over the world and especially in the United States [4, 5]. Obesity is the accumulation of fat tissues in the intra-abdominal region which is linked to metabolic abnormalities such as insulin resistance, contributing to the pathogenesis of type 2 diabetes mellitus [6]. Diabetes mellitus is caused when insulin function is impaired in metabolic tissues, and this state is known as insulin resistance. Insulin resistance can also be caused when the sensitivity of the insulin receptors is altered. Use of pharmacological agents like the antagonist or the inverse agonist of the cannabinoid (CB_1) receptor, rimonabant, can help counteract impaired insulin receptor sensitivity in the body [7]. Therefore, obesity is strongly linked to insulin resistance and diabetes mellitus [6], and as skeletal muscles play an important role in maintaining glucose homeostasis in the body [8], modulating cannabinoid receptors can help counteract obesity and improve glucose metabolism. This is because rimonabant (RIM) is known to reduce body weight as well as HbA1c levels in diabetic patients [9]. A study found that administration of 3 mg/kg arachidonyl-2-chloroethylamide (ACEA) or 10 mg/kg anandamide (AEA) to function as a CB_1 receptor agonist in a glucose tolerance test conducted in rats demonstrated increase in levels of circulating glucose [10]. Furthermore, use of CB_1 receptor antagonists demonstrated enhanced anti-obesity effects in overweight rats as compared to control rats [11]. This is in correlation with the finding that endocannabinoid levels are found to be increased in central and peripheral organs of experimental animals [12, 13].

As the endocannabinoid system is strongly implicated in both obesity and diabetes, antagonists of this system can be used as potential therapeutic interventions for both these conditions. Both animal and human studies have indicated an increase in endocannabinoids during obesity, especially in peripheral tissues and the hypothalamus [13-15]. Additionally, there was also an increase in the levels of 2-arachidonoylglycerol (2-AG) and AEA in visceral adipose tissues of patients suffering from diabetes and obesity [14, 16, 17]. Studies on CB_1 knock-out mice demonstrated resistance against obesity induced by diet [15, 18]. Initially, antagonism of the CB_1 receptor was considered to mediate hypophagic effects which could lead to weight loss [13, 19]. It was later discovered that, in addition to this, CB_1 receptor antagonism could also enhance metabolic parameters such as increasing glucose tolerance [10, 21], increasing uptake of glucose in skeletal muscles [20],

decreasing hyperinsulinemia [22], and causing an increase in triglycerides and HDL/LDL ratio [23].

Skeletal muscle is considered to be an endocrine organ due to its ability to release inflammatory mediators such as interleukin-6 that can increase glucose metabolism in the muscles in the resting state [24]. Studies have shown increased levels of interleukin-6 in both obesity and diabetes [25]. Furthermore, IL-6 has also demonstrated the capability of increasing both fatty acid oxidation and glucose transport in L6 myotubes [26]. Studies on rat pancreatic acini have indicated that the cannabinoid agonist, WIN55212, inhibits the release of IL-6 [27]. On the contrary, the CB_1 receptor agonist, anandamide was found to enhance the production of IL-6 in astrocytes that were infected with Theiler's murine encephalomyelitis virus [28]. Previous studies have demonstrated a CB_1-dependent increase of IL-6 levels in skeletal muscle cells. When all these findings are considered together, it can be determined that activating or inhibiting the CB_1 receptor can modulate the secretion of inflammatory cytokines such as IL-6 in the myotubes of skeletal muscles, which can, in turn, modulate the metabolism of glucose and fatty acids. This implies that modulating IL-6 in skeletal muscles can lead to an improvement in the symptoms of both diabetes and obesity.

As stated above, the metabolism of glucose and fatty acids mainly takes place in skeletal muscles [29]. Both the mRNA and protein levels of CB_1 receptor have been detected in myotubes and skeletal muscle tissues in both humans and rodents [30, 31]. Additionally, when mice were fed with a high fat diet (HFD), increase in levels of CB_1 receptor in skeletal muscles was detected [30]. CB_1 receptor is mainly expressed in skeletal muscles [30, 31] and its ligands have been shown to reduce cAMP levels in different regions of rat brains [33] and in transfected CHO cells [32] in a CB_1 receptor-dependent manner. Furthermore, CB_1 receptor can activate IL-6 and nuclear receptor subfamily 4, group A (NR4A) in myotubes of skeletal muscles in rats, indicating that it can potentially influence gene expression of HKII, PDK4, ACCB, PFK, PGC1α, and CPT1B in these cells. Therefore, by the upregulation of specific genes, CB_1 receptors can modulate glucose oxidation and insulin resistance (PDK4), glycolysis (HKII and PFK), oxidative metabolism and mitochondrial biogenesis (PGC1α), and fatty acid oxidation and transport (ACCβ, CPT1B, and LCAD) in skeletal muscles. Several studies have provided evidence for this by

demonstrating that 1) the levels of NR4A are decreased in the skeletal muscle tissues of hyperglycemic animals [34], 2) NR4A plays important roles in the utilization of glucose and fatty acids in the body [35], 3) following administration of a high fat diet, NR4A-null mice demonstrate reduced mRNA levels of GLUT4, defects in the phosphorylation of insulin receptor substrate 1 (IRS-1) giving rise to insulin resistance in skeletal muscles, and slow clearance of blood glucose, decrease in energy usage, and increase in body weight as compared to wild-type models [36], 4) studies in C2C12 siRNA-NR4A cells demonstrated the decrease in mRNA levels of fatty acid translocase (CD36/fat), GLUT4, and uncoupling protein-3 (UCP3) when compared to their wild-type counterparts [37], and 5) an increase in the uptake of glucose without the influence of insulin was demonstrated in C2C12 cells engineered for expression of NR4A expression under the control of an adenoviral promoter as compared to wild-type C2C12 cells [38]. Considering all these studies together, using suitable ligands to modulate the CB_1 receptor can influence the utilization of glucose and fatty acids in skeletal muscles. This implies that CB_1 receptor antagonists can prove to be a potential therapeutic intervention for diabetes and obesity and this class of compounds should be investigated for its relevance and suitability.

From the above discussion, agonist and antagonist actions on the CB_1 receptor can influence obesity, inflammation, myogenesis, and metabolism of glucose and fatty acids. Therefore, the aim of this project will be to investigate the signalling events involved in CB_1 receptor activation and inhibition in L6 skeletal muscle cells of rats. Additionally, this work will also investigate the effects of ACEA, RIM, and antagonists, selective agonists, and inverse agonists of CB_1 receptor on gene expression of products involved in metabolic pathways of fatty acids and glucose, and also obesity in L6 skeletal muscle cells of rats.