1. Introduction

Over the recent decades, the prevalence of type 2 diabetes has reached epidemic proportions, and both obesity and insulin resistance (IR) are key characteristics in the development of T2D (Jocken et al., 2007; Olefsky et al., 1973). Insulin resistance is clinically defined as the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in cells (Hojlund, 2014; Lebovitz, 2001). Skeletal muscle accounts for 60-85% of insulin-stimulated glucose disposal and is the most important tissue in regards to peripheral glucose uptake (DeFronzo, 1988; Richter et al., 1989). Oversupply of dietary fat leads to obesity and accumulation of lipids in skeletal muscle (Helge et al., 2001; Starling et al., 1997; Zderic et al., 2004), which is associated with impaired insulin sensitivity (Forouhi et al., 1999; Goodpaster et al., 1997; Storlien et al., 1991).

Lipid is stored as triacylglycerol (TG) in Lipid Droplets (LDs) and expressed ubiquitously in cells including muscles, and nearly all cells have the capacity to store and synthesize LDs (Brasaemle, 2007; Farese and Walther, 2009; Murphy, 1993). LDs differ in size depending of the energy state of the cell (Alsted et al., 2013; Krahmer et al., 2011; Paar et al., 2012; Wilfling et al., 2013). During situations with low energy availability, LDs shrink and fatty acids (FA) are liberated by lipolysis of TG. During lipolysis, lipases act in sequence to catabolize triacylglycerol (TG), in which each step releases one FA. Adipose triglyceride lipase (ATGL) hydrolys the first ester bond releasing FA and diacylglycerol (DAG). DAG is then hydrolysed by hormone-sensitive lipase (HSL) releasing monoacylglycerol (MAG) and FA and, in the last step, MAG is hydrolysed by monoacylglycerol lipase (MAGL) releasing FA and glycerol (Zechner et al., 2009).

HSL and MGL were identified in the early 1960’s (Vaughan et al., 1964), and first thought to be the main lipases responsible for lipolysis of TG. However, DAG accumulates in HSL Knock Out (KO) mice in adipose-, muscle- and testis tissues in response to fasting (Haemmerle et al., 2002; Osuga et al., 2000) and illustrates the requirement for an alternate lipase responsible for the hydrolysis of TG. In 2004, Rudolf Zechner and co-workers, among others, identified ATGL as the main TG lipase (Jenkins et al., 2004; Villena et al., 2004; Zimmermann et al., 2004). The discovery of ATGL led to a revision of the lipolytic pathway and how lipolysis is regulated.

The importance of TG lipases in skeletal muscle has been questioned, as the total hydrolase activity is significantly lower in skeletal muscle compared to adipose tissue. However, when related to the total TG content, the total hydrolase activity is actually 10 times higher in skeletal muscle (Langfort
et al., 1999), clearly illustrating the importance of lipolysis in skeletal muscle, an understanding of how it is regulated. The majority of the studies evaluating regulation of lipolysis have been performed in adipose tissue and cell systems, and regulation of lipolysis is, assumed to be, similar in skeletal muscle.

Different factors such as hormones, adipokines and cytokines have been shown to regulate lipolysis in both adipose tissue and skeletal muscle, of which adrenalin is the most potent and well-described activator of lipolysis in both adipose tissue and skeletal muscle (Ahmadian et al., 2011; Donsmark et al., 2005; Haemmerle et al., 2006; Langfort et al., 2003; Langfort et al., 1999; Watt et al., 2004b; Zimmermann et al., 2004). The energy sensor 5' adenosine monophosphate-activated protein kinase (AMPK) is activated during exercise (Chen et al., 2000; Fujii et al., 2000; Kahn et al., 2005; Stephens et al., 2002; Watt et al., 2006; Wojtaszewski et al., 2000) and electric stimulation of skeletal muscle (Ihlemann et al., 2000; Winder and Hardie, 1996), and has been linked to the regulation of lipolysis. However, whether AMPK is an activator or inhibitor of lipolysis in skeletal muscle is not clear. Accordingly, the first aim of this PhD is to investigate the role of AMPK in the regulation of lipolysis and lipid metabolism during muscle contractions in skeletal muscle.

Lipid is, in muscle cells, stored as intramuscular triacylglycerol (IMTG). It has previously been believed that lipid-induced insulin resistance is due to the accumulation of IMTG (Krassak et al., 1999; Pan et al., 1997). However, recent studies indicate that IMTG does not cause insulin resistance per se, as higher levels of IMTG have been observed in endurance-trained athletes also having a higher insulin sensitivity – this is also known as the “athlete paradox” (Goodpaster et al., 2001; van Loon et al., 2004). In addition, IMTG level is higher in female versus matched male subjects (Steffensen et al., 2002), though, despite this, women are actually more sensitive to insulin than matched men (Hoeg et al., 2009; Nuutila et al., 1995).

During the last decade the lipid intermediates DAG (Shulman, 2014) and Ceramides (Adams et al., 2004) have received increased attention in regard to lipid-induced insulin resistance and in its function as an important second messenger involved in intracellular signaling. Accumulation of DAG is believed to have a detrimental effect on insulin signaling and sensitivity via its ability to activate Protein Kinase C (PKC)ε and (PKC)θ leading to phosphorylation and inhibition of Insulin Receptor Substrate 1(IRS1) and subsequent inhibition of the insulin-signaling cascade in both liver (Samuel et al., 2004; Samuel et al., 2007) and skeletal muscle (Griffin et al., 1999; Li et al., 2004; Yu et al., 2002). However, several studies have not been able to associate the accumulation of
muscle DAG with impaired insulin signaling and insulin resistance (Amati et al., 2011; Hoeg et al., 2011; Vistisen et al., 2008) and thus, questioning the role of DAG as an important mediator of lipid-induced insulin resistance and type 2 diabetes. However, DAG appears in different stereoisomers depending on their origin and cellular localization (Eichmann et al., 2012) and has different reactivity in vitro (Boni and Rando, 1985; Rando and Young, 1984). Thus, the role of DAG in lipid-induced insulin resistance in skeletal muscle may be dependent on the cellular localization and stereo chemical structure of DAG. Accordingly, the second aim of this PhD is to investigate the role of dys-regulation of lipolysis in insulin resistance and if the described function of DAGs as mediators of lipid-induced insulin resistance is dependent on the origin and presence of the different DAG isomers.

The focus of this PhD is to discuss the relevant literature examining the regulation of lipid storage, lipolysis, and the role of lipolysis and DAG on insulin resistance in skeletal muscle.