4. Intro

Diabetes, with a steadily increasing prevalence in past decades, is one of four priority non-communicable diseases targeted for action by world leaders (WHO, 2016). Although exact global estimates of the prevalence of the different types of diabetes do not exist, type 2 accounts for the vast majority (Collaboration, 2016). Type 2 diabetes is a result of inadequate response to insulin (Petersen & Shulman, 2018). A key target tissue for insulin is skeletal muscle, which, in response to insulin, serves as the primary glucose sink of the body (DeFronzo & Tripathy, 2009). During a hyperinsulinemic euglycemic clamp, ~75-80% of the injected glucose is disposed into muscle (Baron, Brechtel, Wallace, & Edelman, 1988; DeFronzo, Gunnarsson, Bjorkman, Olsson, & Wahren, 1985). Insulin-stimulated glucose uptake is highly susceptible to insulin resistance and blunted insulin-induced muscle glucose uptake is indeed an early hallmark in the development of type 2 diabetes (Abdul-Ghani & DeFronzo, 2009). A comprehensive understanding of the processes regulating muscle glucose uptake in response to insulin, insulin-independent stimuli such as exercise/contraction and insulin resistance thus carries considerable potential for development of novel diabetes therapeutics.

To facilitate glucose transport, insulin induces insertion of the glucose transporter (GLUT)4 in the surface membrane of the skeletal muscle fibers (Klip, Ramlal, Young, & Holloszy, 1987). Throughout the thesis increased GLUT4 surface membrane content will be referred to as GLUT4 translocation. Insulin-stimulated GLUT4 translocation is impaired in the insulin resistant state (Etgen, Wilson, Jensen, Cushman, & Ivy, 1996; Garvey et al., 1998; Zierath et al., 1996) and improved by exercise (Hansen, Nolte, Chen, & Holloszy, 1998). GLUT4 translocation can also be stimulated insulin-independently, e.g. by contractions, via different molecular signaling mechanism(s) (Lund, Holman, Schmitz, & Pedersen, 1995). Remarkably, muscle-specific knockout of GLUT4 in mice completely blocked insulin- and contraction-induced muscle glucose uptake and reduced basal glucose uptake by ~80% (Zisman et al., 2000). Thus, GLUT4 translocation is a key process for muscle glucose uptake. Yet, we do not know much about how GLUT4 traffics to reach the surface membrane. This is particularly true in the context of adult and/or human skeletal muscle fibers since most work on GLUT4 trafficking has been carried out in cell culture.

In this PhD project, the aim was to improve our understanding of intramyocellular GLUT4 trafficking and distribution in adult skeletal muscle. The project consisted of 2 individual studies as well as a validation of an assay for measuring in vivo GLUT4 translocation, which will be referred
to as Study 3. Figure 1 presents an overview of the two main studies. The specific aims and hypotheses were the following:

**Study 1:** Skeletal muscle microtubules (MTs) mediate GLUT4 trafficking in adult skeletal muscle and may contribute to insulin resistance.

**AIM:** To investigate the role of the MT network in intracellular trafficking of GLUT4, as well as the susceptibility of the MTs to insulin resistance induced by ceramides.

**Hypothesis:** The MT network constitutes an important means for GLUT4 trafficking in adult skeletal muscle. Insulin induces changes in the MT dynamics and the intracellular trafficking of GLUT4 along MTs. Lastly, C2-ceramide treatment disrupts MT polymerization.

**Study 2:** Prior exercise in humans redistributes intramuscular GLUT4 and enhances insulin-stimulated sarcolemmal and endosomal GLUT4 translocation.

**AIM:** To investigate the intracellular localization of GLUT4 in human muscle following exercise and subsequent insulin stimulation.

**Hypothesis:** Prior exercise induces intracellular redistribution of GLUT4 and increases GLUT4 translocation in response to subsequent insulin stimulation.

**Study 3:** Electroporated GLUT4-7myc-GFP detects *in vivo* glucose transporter 4 translocation in skeletal muscle without discernible changes in GFP-patterns.

**Aim:** To develop an assay for *in vivo* GLUT4 translocation in adult skeletal muscle.

**Hypothesis:** Exercise and AMPK activation by 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) stimulation *in vivo* induce GLUT4 translocation, which can be detected by the GLUT4-7myc-GFP assay, as changes in GFP localization and increased extracellular myc content.
The overall aim of the studies was to gain novel insight into the mechanisms causing GLUT4 translocation. Study 1 investigated the role of the microtubules (MTs) in GLUT4 trafficking and the susceptibility of the MTs to insulin resistance. Study 2 investigated exercise-induced redistribution of GLUT4 between compartments as a potential insulin-sensitization mechanism. In addition to Study 1 and 2, an assay for measurement of in vivo exercise-induced GLUT4 translocation was validated (Study 3).
In the following thesis, GLUT4 trafficking as a whole, including GLUT4 translocation, and its importance for glucose transport in healthy and diseased skeletal muscle will be discussed. This will be based on the current literature, novel observations in human and mouse muscle made in Study 1, 2 and 3 as well as some additional unpublished observations. Specifically, the first part of the thesis will introduce the involvement of GLUT4 in skeletal muscle glucose transport and our current knowledge on GLUT4 trafficking. Secondly, the involvement of the MTs in GLUT4 trafficking will be discussed, and the MT-related data from study 1 put into perspective. Thirdly, the post-exercise insulin-sensitization phenomenon and the concept of intracellular GLUT4 redistribution as a potential component in this process will be discussed, and the human GLUT4 redistribution data from study 2 will be set into perspective. Finally, I will outline some of the technical challenges associated with the measurement of GLUT4 translocation, including the GLUT4-7myc-GFP method used in Study 3 as well as other methodological considerations relevant to this PhD study.