1. Introduction

The worldwide prevalence of type 2 diabetes (T2D) in the adult population has risen from 4.7% in 1980 to 8.5% in 2014, and is estimated to be the seventh leading cause of death by 2030 (WHO). At the same time, the prevalence of obesity has reached pandemic proportions. Caloric excess and energy-dense diets have been ascribed as being among the underlying factors. In this context, an excessive lipid storage in skeletal muscle and liver is observed in association with obesity and proposed to be related to peripheral insulin resistance and dysregulation of hepatic glucose production, culprits in the pathogenesis of T2D.

In prevention and treatment of insulin resistance, diet has been a main focus. Already by 1934, Himsworth et al. had pursued the idea that the relative intake of fat and carbohydrate had an impact on oral glucose tolerance (Himsworth 1934). It was shown in young healthy men that seven days of a high-carbohydrate and low-fat diet induced lower blood glucose excursions after the intake of 50 g glucose when compared to a diet very low in carbohydrates and rich in fat (Fig. 1). Additional experiments led Himsworth to the conclusion that dietary carbohydrate content, rather than fat content, was the main regulator of glucose tolerance.

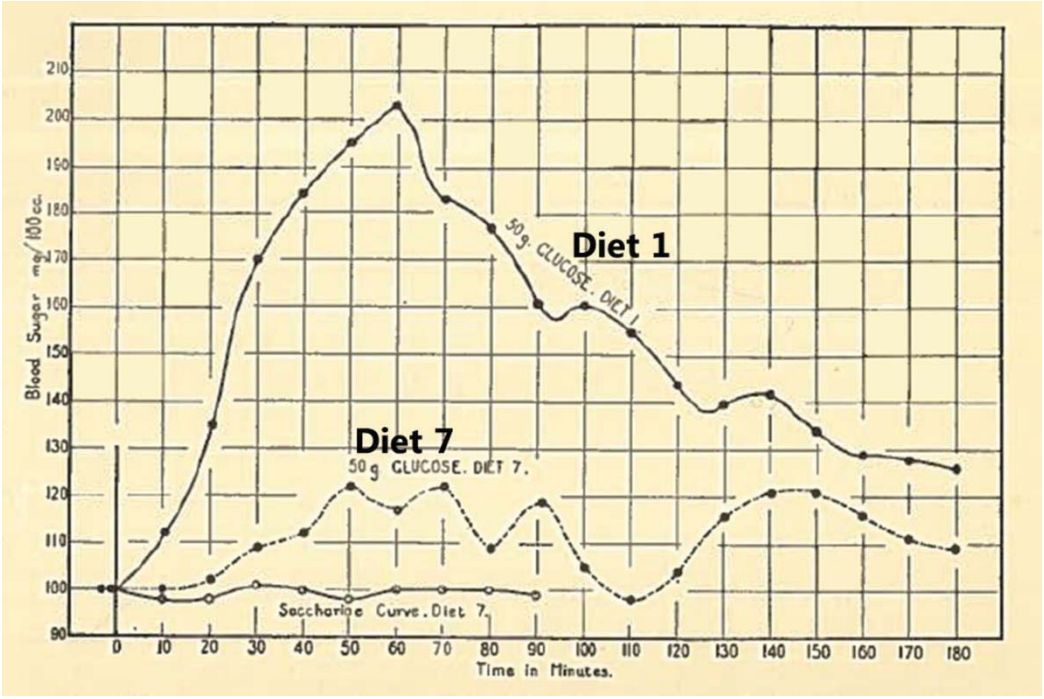

Figure 1. Blood sugar curves after an oral glucose tolerance test, obtained from Himsworth et al. (1934). Data are from one subject. Diet 1: 50 g carbohydate and 240 g fat. Diet 7: 500 g carbohydrate, 40 g fat. Diets were ingested for seven days under weight maintenance.

To date, conclusions on the role of dietary fat in the regulation of insulin and glucose homeostasis remain elusive, as challenged by divergent findings in epidemiologic surveys and intervention studies. This may, in part, be related to eventual lack of adequate study designs or dietary control, and the use of different metrics across studies, i.e. fasting glucose, fasting insulin, HbA1c, oral or intravenous glucose tolerance, intravenous insulin sensitivity or insulin secretion. Earlier studies have pointed that a high fat intake, and in particular a high intake of saturated fat, would negatively influence insulin and glucose homeostasis. When the sum of the recent literature is considered, it can be questioned whether total fat intake or intake of specific dietary fatty acids (FA) are associated with insulin resistance.

The skeletal muscle glucose metabolism serves as a critical regulator of whole-body glucose homeostasis, as skeletal muscle is responsible for ~75% of the glucose disposal during hyperinsulinemic-euglycemic conditions (Baron *et al.* 1988). The specific molecular mechanisms of reduced insulin action in skeletal muscle remain unknown. Alterations in muscle lipid metabolism have gained increasing interest, and have challenged the glucose-centric view on diabetes. Early on, Randle et al. (1963) described the so-called “FA syndrome”, suggesting that increased FA availability in isolated rat heart and diaphragm increased FA oxidation at the expense of glucose oxidation, which led to inhibition of glycolytic enzymes and in turn glucose-6-phosphate (G6P) accumulation and inhibition of glucose uptake. That intramuscular G6P accumulation plays a causal role was, however, challenged by later lipid infusion studies (Roden *et al.* 1996), pointing towards the idea that the defect resides at the step of glucose uptake. In the chase for the culprit in lipid-induced insulin resistance, long-chain fatty acyl-CoA, diacylglycerol (DAG) and ceramide in skeletal muscle have received considerable attention. While initial studies focused on total content of DAG, the research has now expanded to the saturation and chain length of the two acyl chains in DAG.

The intravenous lipid infusion model has been a mechanistic approach to elucidate the molecular mechanisms in lipid-induced insulin resistance. Using this model, Itani et al. (2002) demonstrated, in healthy subjects, that lipid infusion increased total DAG content and protein kinase C (PKC) activity in skeletal muscle, concomitant with reduced whole-body insulin sensitivity (Itani *et al.* 2002). The further causal evidence on the role of DAG is gained from FA incubation studies in

muscle cells (Montell *et al.* 2001), lipid infusion and subsequent analyses on rat muscle (Yu *et al.* 2002) and studies in mice liver (Jornayvaz *et al.* 2011; Neschen *et al.* 2005). The idea is that DAG activates novel PKC isoforms which, in turn, decrease PI3K activation and hence Akt-mediated insulin signaling. Whether these mechanisms are responsible in human skeletal muscle has been challenged by several studies demonstrating no attenuation of insulin signaling after lipid infusion in healthy subjects (Gormsen *et al.* 2007; Hoeg *et al.* 2011; Pehmoller *et al.* 2012; Tsintzas *et al.* 2007; Dube *et al.* 2014; Gormsen *et al.* 2011). This points to the importance of further research into the molecular adaptations to lipid excess in human skeletal muscle. As administration of a lipid emulsion together with heparin acutely raises plasma FA concentration to supraphysiologic concentrations, and thereby bypasses chylomicron secretion and incretine effects, a more physiologic approach would be to increase lipid availability through the diet.

The overall aim of the present PhD project was therefore to investigate the role of dietary fat content and FA quality on whole-body and peripheral insulin sensitivity, hepatic glucoregulation, health-related parameters and molecular metabolism in skeletal muscle. This was investigated in two separate studies in healthy and non-obese subjects. In the present thesis, the main findings in study I and II are summarized, while at the same time reviewing the available evidence from human studies that have manipulated with fat intake.