Interactions between hepatic glucose and fat metabolism in animal models of insulin resistance

Wiegman, Cornelis Harm

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Chapter 1

General Introduction
1. INTRODUCTION

Insulin resistance is defined as the inability of the body to respond adequately to physiological levels of circulatory insulin. The Metabolic Syndrome (1), also named Insulin Resistance Syndrome (2) or Syndrome X (3), consists of a cluster of metabolic and physiologic risk factors that predict the development of type 2 diabetes and cardiovascular diseases. They include insulin resistance, hyperinsulinemia, glucose intolerance, overall and central obesity, hyperlipidemia and hypertension (1,4). Insulin resistance and its consequence, hyperinsulinemia, are considered to represent the shared features that join the obese, hyperlipidemic, glucose intolerant and hypertension phenotypes (5).

The Metabolic Syndrome is highly associated with the Western lifestyle and its prevalence is increasing dramatically in industrialized countries. In 1985 it was estimated that 30 million people worldwide had frank diabetes and this number has increased enormously to 135 million in 1995. The World Health Organization (WHO) predicts an increase to 300 million diabetes patients by the year 2025.

Work described in this thesis is concentrated on the disturbances in glucose and fat metabolism that are associated with insulin resistance. This introduction gives a summary of the current understanding and knowledge of diabetes mellitus and insulin signaling in relation to hepatic glucose and fat metabolism.

2. DIABETES MELLITUS

Three forms of diabetes mellitus can be distinguished. Type 1 diabetes mellitus, also known as insulin-dependent diabetes, is caused by (a) defect(s) in pancreatic function leading to an inability to produce sufficient amounts of insulin. This type of diabetes is often already diagnosed at early age and treatment consists of daily insulin injections with or without oral hypoglycemic drugs and diet. Type 2 diabetes mellitus is also known as maturity onset diabetes and non-insulin dependent diabetes mellitus (NIDDM), although about 30% of these patients are treated by insulin. This type of diabetes accounts for ~90% of all cases and is therefore the most common type of diabetes. Type 2 diabetes is a result of an acquired inability of the body to adequately respond to insulin that is produced upon a physiological stimulus. Because development of the insulin resistant condition is slow and progressive, most type 2 diabetes patients are adults. However, the current senescent life style combined with ingestion of a high-energy diet by teenagers and adolescents has led to an enormous increase in the incidence of type 2 diabetes in this age group (6,7). The third type of diabetes is Maturity-Onset of Diabetes of the Young (MODY) and is a disorder with a clear genetic basis and an autosomal dominant mode of inheritance. MODY can be caused by mutations of several genes: hepatic nuclear factor-4α (MODY1), glucokinase (MODY2), hepatic transcription factor-1 (MODY3), insulin promoter factor-1 (MODY4), hepatic transcription factor-2 (MODY5) and NEUROD1 (MODY6). Still, an estimated 15-20% of MODY patients in Europe do not have mutations of one of the 6 known MODY-related genes (8). A distinct difference between MODY and type 2 diabetes is that MODY patients are not obese and do not always have the Metabolic Syndrome characteristics, like insulin resistance, hyperlipidemia and hypertension, as seen in type 2 diabetes.
3. INSULIN AND INSULIN SIGNALING

Insulin is a 5.8 kDa hormone synthesized by the islets of Langerhans (β-cells) of the pancreas, and was discovered by Frederick Banting and Charles Best in 1921 (9). Insulin contains two polypeptide chains (A and B) which are linked by disulfide bonds (Figure 1, right). Both chains are derived from the same precursor protein named preproinsulin (10) (Figure 1, left). Preproinsulin is synthesized in the endoplasmic reticulum (ER) of the β-cell. The signal sequence (ss) directs the protein into the ER and through the Golgi apparatus to secretory granules where preproinsulin is converted into proinsulin. Enzymes involved in these conversions are the prohormone convertases-2 and -3 (PC2 and PC3) (11-13). In the precursor forms, the amino terminal of chain A and the carboxy terminal of chain B are connected by a third part of the protein, which is named C-peptide. When this part is removed, the two remaining chains form insulin (14,15). Mutations in the insulin gene are rare and by no means account for the enormous number of diabetic patients seen worldwide.

![Figure 1. Conversion of preproinsulin into proinsulin and insulin. A = A chain, B = B Chain, C= connecting peptide, ss = signaling sequence.](image)

Insulin is best known for its physiological role in maintenance of glucose homeostasis but also has important regulatory roles in fat metabolism and protein synthesis. A signaling cascade is necessary for insulin to exert its metabolic effects. The initial step in the insulin signal transduction pathway is the binding of insulin to its receptor, which is located on the cell surface of different target tissues. The insulin receptor is a heterotetrameric transmembrane glycoprotein and contains 2 α- (130 kDa) and 2 β-subunits (95 kDa), which are connected by disulfide bonds. The α-subunits are located extracellularly and contain the insulin binding domain. The β-subunits consist of extracellular, membrane-spanning and intracellular domains, which contain tyrosine-specific protein kinases (16).

Binding of insulin to its binding domain results in the activation of the tyrosine protein kinase on the intracellular domain of the β-subunits and subsequently results in autophosphorylation of specific tyrosine residues on the β-receptor subunits (16). This activation of the insulin receptor attracts and activates intracellular proteins involved in several signal transduction pathways. The phosphatidylinositol 3-kinase (PI3K) pathway and the mitogen activated protein (MAP) kinase pathway are the two important signal transduction pathways for insulin that are involved in the metabolic effects of insulin on hepatic glucose and fat metabolism (17-19).
3.1 Phosphatidylinositol 3-kinase pathway. The phosphatidylinositol 3-kinase (PI3K) pathway is thought to be the main pathway by which insulin elicits its functions in glucose and fat metabolism (18-20). The signal transduction pathway is schematically displayed in Figure 2.

Due to activation of the insulin receptor, other downstream molecules become activated. The insulin receptor activates the insulin receptor substrates (IRS) by phosphorylation of specific motifs creating high-affinity binding sites for the p85α subunit of PI3K. Binding between IRS and p85α causes allosteric changes in the conformation of the p85α subunit resulting in activation of the p110 catalytic subunit of PI3K (16) and initiates the production of the phosphatidylinositols, i.e., phosphatidylinositol 3,4,5-trisphosphate (PIP3) and phosphatidylinositol 3,4-biphosphate (PI(3,4)P2) (21,22). These molecules are responsible for further signaling in this cascade (Figure 2), involving protein kinase B and C (PKB and PKC), which are possibly involved in positive and negative feedback regulation of the insulin signal (23,24). The insulin signal ultimately results in glucose uptake by promoting translocation of the glucose transporters 2 (GLUT2) to the cell membrane and by stimulation of glycogen.
formation. The key enzyme involved in the latter process is glycogen synthase kinase-3 (GSK-3), which is phosphorylated and thereby inactivated by PKB. This results in dephosphorylation and activation of glycogen synthase (GS) leading to the production of glycogen (Figure 2).

3.2 MAP kinase pathway. The effects of insulin on the regulation of protein synthesis is thought to be mediated by the MAP kinase pathway (Figure 3). After activation of the insulin receptor tyrosine protein kinase, GRB2 becomes phosphorylated (25). GRB2 dimerizes with SOS, which is the guanine nucleotide exchange factor able to activate Ras. Activated Ras is able to initiate a cascade of activated protein kinases called the mitogen activated protein kinases (MAP). GTP-Ras binds to Raf-1, the first kinase in this cascade, which subsequently activates MEK (also called MAP kinase kinase) (26). Activated MEK phosphorylates ERK-1 and ERK-2 (MAP kinases) on tyrosine and threonine residues. The MAP kinases have a wide range of potential substrates including transcription factors and other kinases (reviewed by (23)) influencing protein synthesis (Figure 3).

Figure 3. Insulin signal transduction: the mitogen activated protein kinase (MAP) pathway.
4. HEPATIC GLUCOSE METABOLISM

Glucose is the major energy source for many organs and cells and the sole source for specific cells, including those of the brain. Because maintenance of crucial functions in the body depends on the supply of glucose by the bloodstream, blood glucose levels must be carefully maintained. The liver plays a major role in glucose homeostasis by controlling the balance between glucose uptake and output during sequential periods of feeding and fasting. Postprandially, the insulin-mediated glucose uptake yields glucose that can be either stored as glycogen (glycogenesis) or oxidized to meet energy demands (glycolysis). During fasting, glucose is produced by degradation of glycogen (glycogenolysis) or synthesized using alternative substrates like glycerol, lactate and certain amino acids (gluconeogenesis).

4.1. Metabolic pathways. Intracellular glucose can have different fates in the hepatocyte, depending on the prevailing metabolic conditions. During and after feeding, glucose enters the hepatocyte and is either oxidized or stored as glycogen. The first step in hepatic glucose metabolism is its conversion of glucose by glucokinase into glucose-6-phosphate (G6P) (Figure 4, step I). This metabolic intermediate is thought to be one of the important regulators of glucose metabolism (27). G6P can enter different metabolic pathways. It can be oxidized (Figure 4, step II), a multiple-step metabolic pathway ultimately leading to the formation of pyruvate, which can be further oxidized to acetylCoA in mitochondria and enter the Krebs cycle to generate energy and CO₂. Another pathway available for G6P is storage into glycogen (step III). Enzymes catalyzing this pathway, glycogen synthase kinase-3 (GSK-3) and glycogen synthase (GS) are phosphorylated and dephosphorylated, respectively, by the insulin-signaling cascade (PI3K pathway) leading to the formation of glycogen (Figure 2).

![Figure 4](image_url)  
**Figure 4.** Major metabolic pathways in hepatic carbohydrate metabolism. Sharing glucose-6-phosphate as metabolite. These metabolic pathways are: I. Glucose phosphorylation, II. glycolysis, III. glycogen synthesis, IV. glycogenolysis, V. G6P hydrolyses and VI. *de novo* G6P synthesis. The gluconeogenic flux to glucose (gluconeogenesis) is represented by VI + V.
When energy supply from dietary sources becomes limited, like in the fasted condition, glucose can rapidly be mobilized from the glycogen stores. Glycogen is converted into G6P (step IV) by glycogen phosphorylase (GS) and subsequently into glucose by glucose-6-phosphatase (G6Pase) (step V). Glucose is released into the blood to be transported to extrahepatic tissues. However, glycogen reserves are limited. During depletion of glycogen stores, the process of glucose synthesis (gluconeogenesis) becomes accelerated. The precursors are lactate, glycerol and the amino acids alanine and glutamine. These precursors, except glycerol, enter the gluconeogenic pathway via pyruvate and are subsequently converted in G6P. Glycerol enters the pathway as dihydroxyacetone phosphate, an intermediate in this pathway, and is subsequently converted into G6P. The key enzyme in this process is phosphoenolpyruvate carboxykinase (PEPCK). Transcription of the PEPCK gene is regulated by insulin, glucocorticoids and cAMP, in order to adjust glucose production to physiologic requirements.

During insulin resistant conditions, most of the glucose metabolic pathways are affected to a more or lesser degree, depending on their mode regulation. The suppressive effect of insulin on G6Pase and PEPCK expression levels is decreased (28). Glucose uptake is decreased and together with an increased glucose production this will ultimately lead to hyperglycemia (29,30).

Figure 5. Hepatic glucose-6-phosphate/glucokinase cycle.
4.2. Glucose-6-phosphatase/glucokinase cycle. Glucose-6-phosphatase (G6Pase) and glucokinase (GK) are two enzymes critically involved in controlling the rate and direction of the hepatic glucose flux. G6Pase catalyzes the terminal step of both the gluconeogenic and glycogenolytic pathways (31). It is an enzyme which hydrolyses G6P into glucose and organic phosphate (Pi). G6Pase is located in the endoplasmic reticulum (ER) lumen embedded within the ER membrane. The protein complex consists of different functional proteins with transport capabilities and enzyme activities (Figure 5). Glucokinase (also known as hexokinase IV) is the hepatic glycolytic enzyme opposite of G6Pase and catalyzes the conversion of glucose into G6P (32). GK translocates between the nucleus and cytoplasm in response to metabolic change (33). The activity and translocation of GK is inhibited when the GK regulatory protein (GKRP) binds to GK. This binding takes place in the nucleus. In response to elevated plasma glucose, GK is released from GKRP and translocates to the cytoplasm to perform its metabolic actions (Figure 5) (34).

The glucose transporter 2 (GLUT2) facilitates hepatic glucose uptake. GLUT 2 is a member of a family of integral membrane-protein glucose-transporters and primarily expressed in the liver (35). After their uptake, glucose molecules are "trapped" inside the liver due to the conversion into G6P. Only after G6P hydrolysis into glucose and organic phosphate (Pi) and glucose transport back to the cytoplasm, glucose can subsequently be expelled into the blood by GLUT2 (Figure 5).

Regulation of these pathways is important to balance the hepatic glucose uptake and output influencing plasma glucose levels. Glucose-induced insulin secretion normally suppresses G6Pase expression and enzyme activity via the PI3K pathway (36). Also PEPCK activity is suppressed by insulin resulting in reduced glucose production and output (37). Next to the decreased glucose production, insulin induces GK activity leading to increased glucose uptake (38,39). The insulin-induced alterations of expression levels and enzyme activities in these pathways are counter-regulated by glucagon, a hormone, which is often seen as the antagonist of insulin (38). The transcription factor involved in these insulin-mediated effects is the sterol regulatory element binding protein-1c (SREBP-1c). Insulin-induced SREBP-1c expression and activity results in downregulation of PEPCK gene expression and upregulation of GK gene transcription (40-42), resulting in a decreased glucose production and an increased glucose uptake.

In insulin resistant conditions the balance between glucose uptake and glucose production is disturbed; increased glucose output (lower part of Figure 5) and decreased glucose uptake (upper part of Figure 5) leading to hyperglycemia.

5. HEPATIC FAT METABOLISM

The liver plays a central role in the controlled delivery of lipids to peripheral tissues through tightly regulated production and secretion of very-low density lipoproteins (VLDL). VLDL are lipid-containing particles that distribute lipids from the liver to peripheral tissues. The VLDL production process determines, in part, plasma lipoprotein levels and thereby atherogenicity of plasma lipid profiles and is influenced by nutritional conditions and hormonal status. Overproduction of large, triglyceride-rich VLDL is a hallmark of the diabetic phenotype.

5.1 Very-low density lipoprotein formation. The secretion of Very Low Density Lipoproteins (VLDL) makes an important contribution to hepatic lipid balance and hormonal control of this process is essential for an appropriate acute response to intake of dietary fats.
VLDL-particles function as lipid transporters to distribute lipids from the liver to different peripheral tissues like muscle, heart, and adipose tissue for use as fuel or for storage. VLDL-particles consist of a hydrophobic core containing triglycerides (TG) and cholesteryl esters (CE) (Figure 6). Apolipoprotein B (apoB), a large protein (4536 amino acids, 520 kDa) with many, relatively short, hydrophobic stretches of amino acids sequences, associates with these core lipids during formation of the particle in the smooth ER. A monolayer of polar "surface" lipids, including cholesterol, phospholipid and a number of apolipoproteins (apoE and apoC-I, -II, and -III) cover the surface area left unoccupied by apoB.
The most common opinion regarding the process of lipoprotein formation is described as by the "Two-step hypothesis", which was already proposed by Alexander et al. in 1976 (43) and is displayed in Figure 6. The first step of lipoprotein production is the formation of a primordial, lipid-poor apoB-containing, precursor particle of high density, which fuses with a lipid droplet to become a mature lipid-rich lipoprotein (43-45). The "first step" particle is formed in de rough ER. After initiation of translation and translocation of the apoB molecule into the lumen of the ER, the nascent polypeptide folds into a globular protein with amphipathic lipid-binding domains (β-sheets and α-helical domains) (46). Lipidation of these domains stabilizes the apoB protein. The microsomal triacylglycerol transfer protein (MTP) catalyses the transfer of triglycerides and other non-polar lipids, e.g., cholesteryl ester and phosphatidylcholine, toward apoB. MTP is a 97 kDa heterodimer in association with protein disulphide-isomerase, which is essential for the correct folding of apoB (47,48). This results in the release of apoB from the ER membrane and in the formation of primordial lipoproteins (49). It has been suggested that lipidation decreases the interaction between apoB and MTP, and provide signals for the exit of these particles for secretion into plasma (49).

The second step is the fusion of the apoB-containing "first step" particle with an apoB-free lipid droplet. This second step particle is formed in the smooth ER (43) and fuses with the apoB-containing particle formed in the first step. The fusion of the two particles results in the formation of the nascent VLDL-particle, which, after modification in the Golgi apparatus, can be secreted into the blood (Figure 6).

5.2. VLDL processing. VLDL-TG is hydrolyzed by lipoprotein lipase (LPL) and hepatic lipase (HL) into free fatty acids and glycerol, which are taken up by peripheral tissues, and results in the formation of VLDL remnants or IDL particles. These particles are taken up by the liver or hydrolyzed further into LDL particles. A large variety of receptors is present in the liver, intestine, lung, brain and muscle to facilitate the uptake of these particles (50-53). These include the LDL receptor (LDLR), the LDLR-related protein (LRP) and the VLDL receptor (VLDLR) (50-52,54,55). Independent form its catalytic activity, LPL is probably also involved in the interaction of lipoproteins with their receptors (56,57).

Overproduction of VLDL particles by the liver is often seen in human insulin resistant states (58,59) and affects whole body lipid metabolism as well as plasma lipoprotein profile. The association of hyperlipidemia with type 2 diabetes has been studied longitudinally and progressively increases during the development of type 2 diabetes (60). Normally, insulin suppresses VLDL secretion (61,62), but this suppressive effect is lost in the insulin resistant condition seen in obese and type 2 diabetic patients (63). The mechanism(s) behind the inability of insulin to suppress hepatic VLDL production is (are) not entirely known. The inability of insulin to accelerate the intracellular degradation of apolipoprotein B (apoB) results in an overproduction of relatively large VLDL particles (58,59).

Insulin stimulates LPL activity and result in hydrolysis of plasma lipoprotein particles. Regulation of LPL is affected in insulin resistant individuals (64) and may result in impaired or delayed removal of lipoprotein particles which contributes to hypertriglyceridemia. A secondary consequence of the prolonged residence of VLDL particles in the circulation is the exchange of their TG content with cholesteryl ester in HDL and LDL particles, a process facilitated by the cholesteryl ester transfer protein (CETP) (65). This results in the presence of CE-depleted, TG-rich LDL particles. LPL is able to remove TG from these particles leading to the formation of small, dense LDL particles, also called pattern B LDL (66-68). These particles are more susceptible to oxidation and, therefore, constitute an increased risk for development of cardiovascular disease (69-71).
Table 1. Physical properties and composition of human lipoproteins (72,73)

<table>
<thead>
<tr>
<th></th>
<th>Chylomicrons</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Origin</strong></td>
<td>Intestine</td>
<td>Liver</td>
<td>VLDL</td>
<td>VLDL</td>
<td>Liver/Intesti</td>
</tr>
<tr>
<td><strong>Diameter (nm)</strong></td>
<td>75-1200</td>
<td>30-80</td>
<td>25-35</td>
<td>18-25</td>
<td>5-12</td>
</tr>
<tr>
<td><strong>Density (g/ml)</strong></td>
<td>&lt;0.96</td>
<td>0.96-1.006</td>
<td>1.006-1.019</td>
<td>1.019-1.063</td>
<td>1.063-1.210</td>
</tr>
<tr>
<td><strong>Composition (weight%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>88</td>
<td>56</td>
<td>29</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3</td>
<td>15</td>
<td>34</td>
<td>48</td>
<td>30</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>1</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>8</td>
<td>20</td>
<td>26</td>
<td>28</td>
<td>45</td>
</tr>
<tr>
<td>Protein</td>
<td>1-2</td>
<td>6-10</td>
<td>11</td>
<td>21</td>
<td>45-55</td>
</tr>
<tr>
<td>Apolipoproteins</td>
<td>B48, A-I, A-IV, C1, C2, C3, E</td>
<td>B100, C1, C2, C3, E</td>
<td>B100, C1, C2, C3, E</td>
<td>B100</td>
<td>A-I, A-II, E</td>
</tr>
</tbody>
</table>

5.3. Metabolic pathways. The availability of newly synthesized triglycerides in the hepatocyte may be a very important factor in control of VLDL production. There are several sources of fatty acids that are potentially available for the synthesis of hepatic VLDL-triglycerides. De novo lipogenesis, cytoplasmic triglyceride stores, fatty acids derived from lipoproteins taken up by the liver and plasma free fatty acids (FFA) are sources of fatty acids which can potentially be used for VLDL-TG synthesis (74,75).

The process of fatty acid synthesis, de novo lipogenesis (DNL), accounts for the formation of less than 5 gram of fatty acids per day in humans (76). The rate-limiting enzymes involved in the DNL are acylCoA carboxylase (ACC) and fatty acid synthase (FAS). The transcription of genes encoding these enzymes is under control of transcription factors like the sterol regulatory element binding proteins-1c (SREBP-1c) which is activated by insulin. The contribution of newly synthesized fatty acids to VLDL-lipids is only 3-5% in healthy human volunteers (77,78). Newly synthesized fatty acids are potentially a source for incorporation into VLDL particles but can also stored in the liver as triglycerides or esterified into phospholipids or cholesteryl esters (79).

The intracellular cytoplasmic triglyceride stores contributes about 70% of the triglycerides needed for lipoprotein formation in a process that involves lipolysis and re-esterification of this pool of triglycerides. A major proportion of the extracellular fatty acids is esterified and temporarily stored intracellularly before mobilization for VLDL assembly (74). Also the de novo synthesized triglycerides derived fatty acids and/or phospholipids (79) enter this pool and all undergo a process of lipolysis and re-esterification before secreted as lipoprotein-particles. The re-esterified fatty acid that is not destined for secretion returns to the cytoplasmic storage pool (74).

The liver is capable to take up lipoproteins from the circulation. Receptor-mediated-endocytosis is thought to be the mechanism for this process, which involves several different receptors like the LDL receptor and the scavenger receptor. After the hydrolysis of TG and CE in hepatocyte lysosomes, the fatty acids and glycerol can be re-used in the triglyceride and phospholipid synthesis (75).

Another source of free fatty acids comes from the mobilization of FFA from the adipose tissue. Hormone sensitive lipase (HSL) is the key enzyme of intracellular TG hydrolysis and is the major enzyme involved in the fatty acid mobilization in adipose tissue (80). The rate of FFA supply to the liver is thought to be one of the factors controlling VLDL secretion (80-82). Insulin is the most important physiological inhibitor of lipolysis resulting in
increased rate of lipolysis during insulin resistance and an increased FFA flux towards the liver.

Next to storage as TG and secretion into VLDL, lipids can also be oxidized to generate energy. When energy demand is increased and glycogen reserves are depleted, triglycerides are mobilized from lipid stores, hydrolysed by LPL and HSL into fatty acids. The free fatty acids are transported to the liver and enter the β-oxidation cascade. Fatty acid oxidation occurs in the mitochondria, peroxisomes and smooth ER (ω-oxidation) (83-85). Enzymes involved in fatty acid oxidation are under transcriptional control by peroxisome proliferator-activated receptor α (PPARα) (86,87). The result is the formation of energy (ATP) and ketone bodies (3-hydroxy butyrate and acetoacetate) which are utilized as an alternative energy sources by extrahepatic organs, particularly the brain.

Hepatic fat metabolism is important in generating energy and the delivery of various substrates to extrahepatic organs. These processes are highly dependent on the dietary and hormonal status. The major pathways affected by insulin resistance-associated hyperlipidemia are the overproduction of VLDL particles and further processing of these particles leading to an additional risk factor for cardiovascular diseases.

6. INTERACTIONS BETWEEN GLUCOSE AND FAT METABOLISM

Glucose and fat metabolism are closely linked to each other because of the necessity that if glucose oxidation decreases the oxidation of fatty acids increases to meet energy requirements. However, the exact relationship is not certain. In 1963, Randle et al. postulated the glucose-fatty acid cycle (88). Based on experiments in rat heart and diaphragm muscle, the glucose-fatty cycle states that the availability of free fatty acids determines the rate of FA oxidation and that FA oxidation directly inhibits glucose oxidation. The exact mechanism behind this interaction is still under discussion. Several mechanisms have been proposed to explain the glucose-fatty acid cycle, e.g. the inhibition of pyruvate dehydrogenase (glycolytic enzyme) by an increased acetylCoA/CoA ratio, inhibition of phosphofructokinase due an increased in citrate level and the inhibition of hexokinase (glucokinase in liver) mediated by G6P (89).

In humans, there is no direct evidence in support of the original hypothesis postulated Randle et al. (88). In studies in which plasma fatty acid levels were increased by experimental means, no effects were detected on intracellular (muscle) citrate or G6P levels (90-93). During elevated plasma insulin (hyperinsulinemic euglycemic clamp) and elevated FA (lipid emulsion) the rate of glucose infusion to maintain euglycemia was decreased when FA levels were increased (94). This indicates that the availability of FA decreased glucose uptake but does not necessarily indicate a decreased glucose oxidation. When glucose uptake is constant at high rates (hyperinsulinemic hyperglycemic clamp) increased plasma FA levels had no effect on glucose oxidation (95). Controversially, glucose instead of FA might also be the primary determinant of substrate oxidation (96). When the availability of glucose was increased to supraphysiological levels, FA oxidation decreased, despite a constant availability of FA (94). An explanation for this effect might be that glucose (or insulin) directly limits fatty acid oxidation by restricting the uptake of long chain fatty acids into the metabolic pathways (97). Although the precise mechanism of the interaction between glucose and FA metabolism may vary in different circumstances or organs for that matter, it does emphasis the substrate interactions and reciprocal relationships that exist between glucose and fatty acids.

The substrate interactions between glucose and fat metabolism are also seen at the level of gene transcription and protein synthesis of enzymes involved in these metabolic
pathways. Several transcription factors are induced by metabolic substrates (glucose, FA and cholesterol) or metabolites thereof and hormones (insulin and glucagon) and are subsequently able to modulate expression levels of genes encoding enzymes involved in glucose and fat metabolism. Table 2 summarizes some relevant transcription factors, some of their target genes and the affected metabolic pathways.

Table 2. Transcription factors involved in hepatic glucose and fat metabolism.

<table>
<thead>
<tr>
<th>Transcription Factor</th>
<th>Target genes</th>
<th>Glucose metabolism</th>
<th>Fat metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>SREBP-1</td>
<td>ACC, FAS, PEPCK, G6Pase, GK</td>
<td>Glucose production</td>
<td>Fatty acid synthesis</td>
</tr>
<tr>
<td>SREBP-2</td>
<td>HMGCoA reductase and synthase, LDLR,</td>
<td>x</td>
<td>Cholesterol synthesis</td>
</tr>
<tr>
<td>CHREBP</td>
<td>PK, FAS</td>
<td>Glycolysis</td>
<td>Fatty acid synthesis</td>
</tr>
<tr>
<td>PPARα</td>
<td>AcylCoA oxidase, bifunctional enzyme</td>
<td>Gluconeogenesis</td>
<td>Fatty acid oxidation</td>
</tr>
<tr>
<td>PPARγ</td>
<td>LPL, AFABP, acylCoA synthase, FATP, GK</td>
<td>Glucose uptake</td>
<td>Fatty acid storage</td>
</tr>
</tbody>
</table>

The sterol regulatory binding proteins (SREBPs) are membrane bound transcription factors whose active domains are released proteolytically to enter the nucleus to activate genes involved in the synthesis and uptake of cholesterol, FAs and glucose (98). They function by binding on specific DNA sequences called sterol regulatory elements (SRE; ATCACCCCAC) and E-boxes (CAXXTG) in the promotors of their target genes (99). SREBP-1a and -1c isoforms are produced from a common gene that uses alternative promotors and are more associated with regulation of fatty acid and glucose metabolism. SREBP-2 is encoded by a separate gene and is more associated with regulation of cholesterol metabolism (100,101). Both insulin and glucose are able to increase hepatic SREBP-1c expression levels (41,102,103) and thereby induce hepatic lipogenic enzymes. SREBP-1c also regulates adipogenesis by activating PPARγ (104). However, the exact role of SREBP-1c in adipogenesis and lipogenesis in the adipose tissue is not clear. Sterol regulation is the primary mechanism that activates SREBP-2. In the absence of sterols, SREBP-2, which, in complex with the putative cholesterol sensor, SREBP cleavage-activating protein (SCAP), translocates to the Golgi apparatus, where SREBP-2 is cleaved by site-1 protease (105). The mature SREBP-2 protein enters the nucleus and influences genes involved in cholesterol synthesis. Liver cholesterol depletion specifically inhibits the processing of SREBP-1c at the same time that it activates the processing of SREBP-2. HMG-CoA reductase, HMG-CoA synthase, farnesylidiphosphate synthase, and squalene synthase are genes involved in cholesterol synthesis and are under the control of SREBP-2 (100,101). Drugs that lower the expression levels of these SREBP-2 target genes are statins. Statins lower plasma LDL-cholesterol concentration by inhibiting cholesterol synthesis, inducing LDLR expression (106) and reduce the risk of cardiovascular disease (107).

PPARα and PPARγ belong to the nuclear hormone receptor family that are activated by FAs and participate in control of fatty acid metabolism. PPARs bind to specific response
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elements, the peroxisome proliferator response elements (PPREs), which consists of a direct repeat of the AGGTCA sequence separated by 1 nucleotide (DR-1) (108). PPARs bind to the promoter only as a heterodimer with the retinoid X receptor (RXR). PPARα is more associated with the fatty acid oxidation in the liver and PPARγ influences fatty acid storage in adipose tissue (86). However, PPARα is also associated with glucose metabolism. PPARα–null mice have lower plasma glucose concentration after prolonged fasting compared with wild-type mice, indicating that gluconeogenesis is impaired in these mice (109). Insulin is able to induce PPARγ activity (110,111), but this activation may also be mediated by SREBP-1c. Recently, PPARγ was found activate GK in pancreatic β-cells influencing the glucose-sensing apparatus (112). If PPARs have a more profound role in glucose metabolism is not clear. Two synthetic drugs which are used as treatment of diseases, which belong to the Metabolic Syndrome, are fibrates and thiazolidinediones (TZD). Fibrates are hypolipidemic drugs that bind PPARα with high affinity. They lower plasma TG concentration, by stimulating hepatic FA oxidation and apoCIII expression, and increase HDL levels, due to the induction of apoAI and AIII expression (113). TZDs bind PPARγ with a moderate to high affinity and have a hypoglycemic effect. TZDs are thought to divert FA away from skeletal muscle towards adipose tissue and therefore increase insulin-induced glucose uptake in skeletal muscle. Both drugs are used clinically to lower plasma lipids and glucose concentration in type 2 diabetes patients (114).

The carbohydrate response element binding protein (CHREBP) is the transcription factor that binds to carbohydrate-responsive elements (two "half E boxes" CACG) in promotor regions of genes and is expressed specifically in the liver (115,116). This transcription factor is potentially involved in the glucose-induced fatty acid synthesis and might be independent from the effects of insulin on this process (115,117). Overall, regulation of genes involved in glucose and fat metabolism is a complex process with many interactions between different transcription factors, which depends on nutritional and hormonal conditions.

7. OBJECTIVES AND OUTLINE OF THIS THESIS

The studies described in this thesis are focused on the disturbances that occur in hepatic glucose and fat metabolism during insulin resistant conditions in specific animal models. The different metabolic pathways in glucose and fat metabolism are differently affected under these conditions but the interrelationships and underlying mechanisms are not fully understood. To study the consequences of insulin resistance on metabolic pathways, we made use of mass isotopomer distribution analysis (MIDA) to accurately quantify the relevant fluxes of glucose and fat metabolism. This technique makes use of the distribution and quantification of stable isotope-precursors, e.g., [1-13C]-acetate or [2-13C]-glycerol, into molecular species of "biological polymers", such as cholesterol, fatty acids and glucose. In combination with the calculated dilution of the precursor and the size of the polymer pools, kinetic parameters can be calculated, i.e. contribution of endogenous biosynthetic pathways to the total pool and absolute rates of biosynthesis (118). In our studies, these parameters were compared with hepatic and/or intestinal gene expression profiles and enzyme activities of key enzymes involved in these metabolic pathways to develop an integrated picture of the metabolic consequences of insulin resistance.

VLDL production was studied in several animal models described in this thesis since overproduction of large, TG-rich VLDL particles by the liver in insulin resistant conditions is a direct consequence of the inability of insulin to suppress this process. However the exact mechanism(s) behind this inability of insulin is (are) not entirely known.
High-fat feeding in rodents results in the development of insulin resistance (119) and was studied in relation with the effects of insulin on hepatic glucose metabolic fluxes (Chapter 2) and on VLDL production (Chapter 3). To determine the mechanism behind the insulin resistant condition, hepatic insulin signal transduction was also studied.

Next to high-fat diet-induced insulin resistance in rats, two mouse models were studied in which the insulin resistant condition has a genetic basis. The ob/ob mouse model is a well-characterized model of type 2 diabetes (120-123). Hepatic de novo lipogenesis and cholesterol synthesis were quantified in combination with the effects of insulin on hepatic VLDL production (Chapter 4). The second mouse model described in this thesis is the intestinal-fatty acid binding protein knockout mouse (Fabpi−/−). In contrast to the ob/ob mouse model, this model is relatively new and may serve as a model for certain human conditions. Fabpi−/− mice were created by standard gene disruption technology in the laboratory of Dr. L. Agellon (Edmonton, Canada) and showed some diabetic features (124). In these mice the insulin resistant condition was quantified in combination with the effect of insulin on VLDL production (Chapter 5).

Hepatic glucose handling is disturbed in the insulin resistant condition. Glucose-6-phosphatase (G6Pase), in combination with glucokinase (GK), determines the rate and direction of the hepatic glucose flux and are therefore essential in the regulation of blood glucose homeostasis (27). We studied the effects of acute inhibition of this enzyme on hepatic glucose fluxes (Chapter 6) and hepatic de novo lipogenesis and VLDL production (Chapter 7) in relation to expression levels of genes encoding relevant enzymes and regulators in these pathways.

Outcome of these studies is discussed in an integrated fashion in the General Discussion (Chapter 8).

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### LIST OF ABBRIVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CE</td>
<td>cholesteryl ester</td>
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<tr>
<td>DNL</td>
<td>de novo lipogenesis</td>
</tr>
<tr>
<td>ERK1/2</td>
<td>extracellular ligand regulated kinase-1/2</td>
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<tr>
<td>(F)FA</td>
<td>(free) fatty acids</td>
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<tr>
<td>GLUT</td>
<td>glucose transporter</td>
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<tr>
<td>GS</td>
<td>glycogen synthase</td>
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<tr>
<td>GSK</td>
<td>glycogen synthase kinase</td>
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<tr>
<td>GTP</td>
<td>guanine triphosphate</td>
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<tr>
<td>GRB2</td>
<td>growth factor receptor bound protein</td>
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<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
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<tr>
<td>IDL</td>
<td>intermediate density lipoprotein</td>
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<tr>
<td>IRS</td>
<td>insulin receptor substrate</td>
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<td>LDL</td>
<td>low density lipoprotein</td>
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<td>MAP</td>
<td>mitogen-activated protein</td>
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<td>MEK</td>
<td>MAP/ERK kinase kinase</td>
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<td>MTP</td>
<td>microsomal triglyceride transfer protein</td>
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<tr>
<td>N</td>
<td>nucleus</td>
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<tr>
<td>p85/p110</td>
<td>subunits of phosphatidylinositol 3-kinase</td>
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<td>p90rsk</td>
<td>the 90 kDa ribosomal protein S6 kinase</td>
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<td>PH</td>
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<td>PIP3</td>
<td>phosphatidylinositol 3,4,5-trisphosphate</td>
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<td>RAF1</td>
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