Insulin resistance in obese patients with type 2 diabetes mellitus : effects of a very low calorie diet

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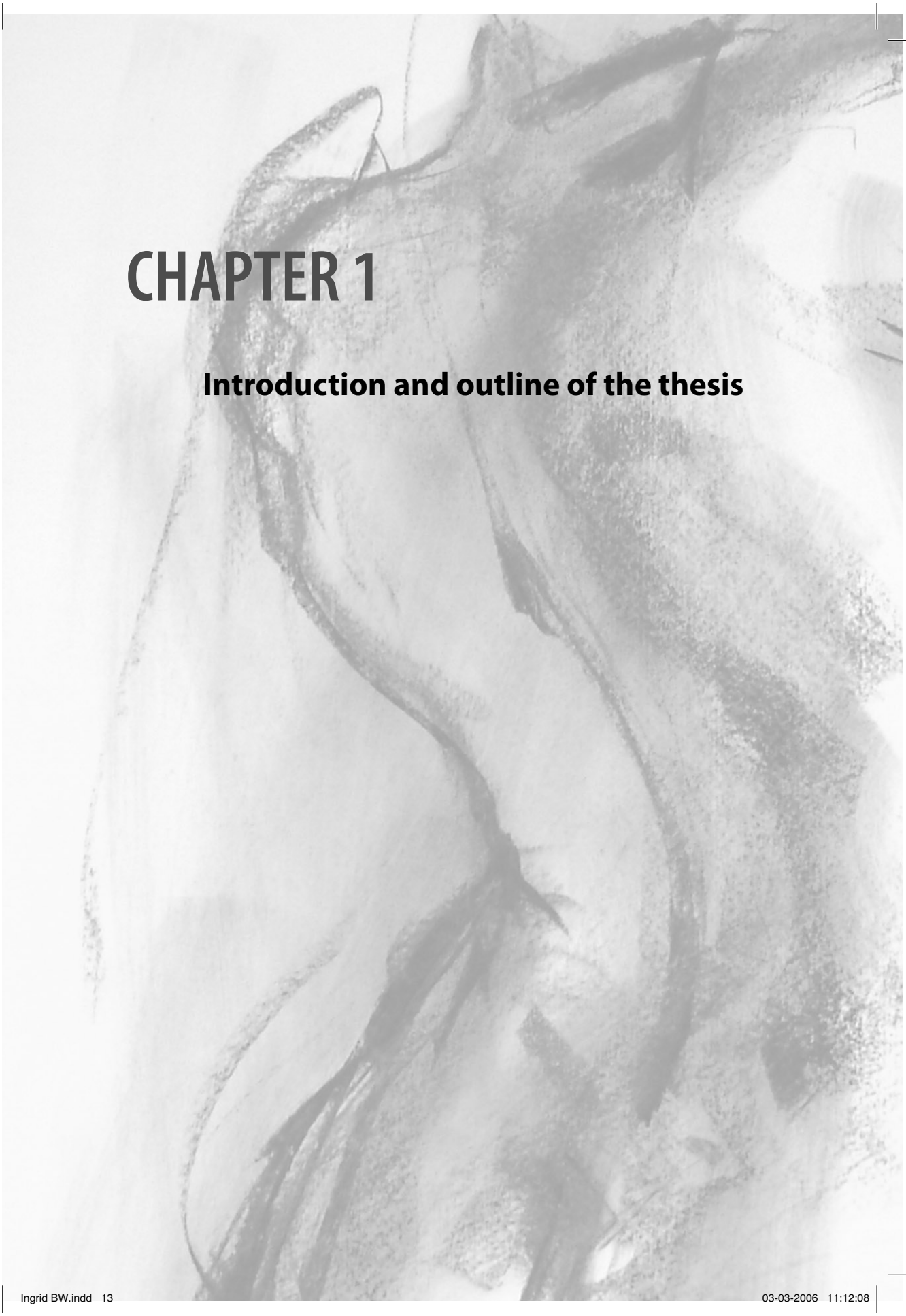

CHAPTER 1

Introduction and outline of the thesis

1.1. OBESITY AND TYPE 2 DIABETES MELLITUS: DEFINITIONS, EPIDEMIOLOGY AND HEALTH PROBLEMS

The enormous increase in overweight and obesity, defined as a body mass index (BMI, calculated as weight in kilograms divided by the length in meters squared) > 25 and > 30 kg/m^2 respectively [Table 1]), has reached epidemic proportions. Worldwide 1 billion people are overweight and 300 million people are obese (http://www/who.int/nut/#obs, obesity and overweight: fact sheet). Of even greater concern is the increase of overweight and obesity in children: worldwide 22 million children under the age of 5 years and 155 million school-age children (http://www.worldheart.org/pdf/press.factsheets.children.obesity.pdf.).

The reason for this concern is that overweight and obesity are associated with increased morbidity and mortality (Tables 2 and 3)[1-4]. Relative risks for the development of type 2 diabetes mellitus[5,6], hypertension[7], coronary heart disease[8,9], stroke[10,11], gallstones[12], osteoarthritis and arthrosis[13,14], infertility[15] and certain types of cancer (breast, colon, endometrium)[16-18] are substantially increased in this patient group (Table 2). Even after correction for diabetes mellitus, high blood pressure and other cardiovascular risk factors, overweight and obesity are in themselves independent risk factors for increased mortality[19]. The association between BMI and mortality has been described as a J-shaped curve with the lowest mortality for BMI values between 18.5 and 24.9 kg/m^2; below 18.5 kg/m^2 the risk is increased and above 24.9 kg/m the risk increases, and rises steeply when the BMI gets over 40 kg/m^2 [20].

Insulin resistance is probably the common denominator, relating obesity with type 2 diabetes mellitus. Obesity somehow (visceral fat deposition?) evokes insulin resistance, a condition predisposing for type 2 diabetes mellitus[21], a chronic disease characterised by impaired insulin secretion and insulin resistance of target organs leading to chronic hyperglycaemia[22]. In fact, in obese women who develop type 2 diabetes mellitus, in 53% of the cases the condition (diabetes) can be ascribed to obesity (Table 2). Therefore, it is not surprising that, along with the increased prevalence of overweight and obesity, the prevalence of type 2 diabetes mellitus has also steadily increased. It is estimated that nowadays over 190 million people worldwide have diabetes mellitus[23], more than 90-95% of them having type 2 diabetes melli-

Table 1. Classification of overweight in adults according to WH01 criteria

Classification	BMI (kg/m2)	Risk of comorbidities
Normal weight	18.5-24.9	average
Overweight	25.0-29.9	increased
Obesity		
Level I	30.0-34.9	moderately increased
Level II	35.0-39.9	severely increased
Level III (morbid)	≥ 40	very severely increased

[1] World Health Organisazation. Obesity: preventing and managing the global epidemic. Technical Report Series,#894,2000.

Table 2. Estimated health risk for obese (BMI ≥ 30 kg/m^2) adults

	Women		Men	
	Prevalence 9.6%*		Prevalence 8.5%*	
	RR	PAR (%)	RR	PAR (%)
Type 2 diabetes	12.7	52.9	5.2	26.3
Hypertension	4.2	23.5	2.6	12.0
Myocardial infarction	3.2	17.4	1.5	4.1
Coloncarcinoma	2.7	14.0	3.0	14.5
Ischemic heart disease	1.8	7.1	1.8	6.4
Gallstones	1.8	7.1	1.8	6.4
Ovariumcarcinoma	1.7	6.3	-	-
Arthrosis	1.4	3.7	1.9	7.1
Stroke	1.3	2.8	1.3	2.5

Prevalence rates concerning obesity are derived from the MORGEN-project RIVM, Int J Obes Rel Metab Dis 2002:1218. The relative risks (RR), are derived from "Tackling Obesity in England. Report by the comptroller and auditor general. London: National Audit Office 2001". This table was derived from the Executive Summary: obesity and overweight, Health Council of the Netherlands, 2003. PAR = population attributable risk, i.e part of the disease that can be attributed to obesity.

Table 3. Body mass index and relative risk of death.

BMI	Relative risk of death
25.0-26.9	1.3
27.0-28.9	1.6
29.0-31.0	2.1

tus. It has been predicted that in the year 2030 366 million subjects worldwide will suffer from diabetes mellitus[24]. These are crude estimates, however, that have not taken into account the increase in overweight and obesity; hence, actual numbers may even be much higher.

Genetic factors are without doubt of major significance in the development of obesity and type 2 diabetes mellitus. However, because the human genome does not change over just decades, genetic predisposition cannot explain the explosive increase in obesity and type 2 diabetes mellitus of recent years. Environmental and social factors, like a lack of physical exercise and high caloric intake, are more likely explanations for the epidemic. A chronic imbalance between energy intake and energy expenditure eventually leads to obesity.

In obese and obese type 2 diabetic patients, insulin resistance is of paramount pathogenetic significance[21,25]. Insulin resistance not only impairs glucose homeostasis, but is also associated with hypertension[26-28], dyslipidaemia[29-31] and abnormalities in coagulation and fibrinolysis[32,33], conditions that are independent cardiovascular risk factors[34-38], seen in both obesity and type 2 diabetes. In addition, insulin resistance in (severely) obese type 2 diabetic patients makes it often difficult to achieve adequate glycaemic regulation. Sooner or later, insulin therapy will be instituted because normalisation of plasma glucose levels cannot be achieved with oral blood glucose-lowering agents alone. Insulin, however, induces weight gain[39], which in turn aggravates insulin resistance, thus requiring higher doses of insulin: a

vicious circle has arisen. Furthermore, insulin therapy can also induce or aggravate already existing hyperinsulinaemia, which could be an independent cardiovascular risk factor[37,38,40,41], although the relation may be week[42].

Weight reduction improves insulin resistance and its associated metabolic features (hypertension, dyslipidaemia, hyperglycaemia)[43,44]. In obese patients this will lead to a lower risk for associated co-morbid conditions (Table 2). It has also been demonstrated that lifestyle intervention programmes (often combinations of behaviour therapy, diet therapy and exercise) in overweight and obese patients reduces the number of patients that develop type 2 diabetes mellitus[45,46]. In severely obese type 2 diabetic patients weight loss is, in fact, the only reasonable therapeutic approach. By reducing insulin resistance, glycaemic regulation can be restored often with much less blood glucose-lowering medication.

Calorie restriction remains the hallmark for weight loss. However, only substantial caloric restriction or more moderate caloric restriction for a longer period of time, will lead to the considerable weight loss (probably > 15 kg[47]) needed to restore peripheral insulin sensitivity in morbidly obese patients and (severely) obese type 2 diabetic patients[47,48]. This can either be achieved through a very low calorie diet (VLCD) or bariatric surgery. The latter is very effective in improving insulin resistance and associated cardiovascular risk factors[43,49-53]. In addition, bariatric surgery can prevent the development of type 2 diabetes mellitus[43,54] (review bariatric surgery:[56,57]). However, the procedure is invasive, costly and (also for logistic reasons) available for a limited number of subjects only. VLCDs are safe[58], commercially available, relatively cheap, and easy accessible. Given the enormous increase in incidence of obesity and (obese!) type 2 diabetes mellitus, VLCDs are, therefore, an interesting therapeutic option. Thus, the main focus of the studies described in this thesis was to investigate the short-term and long-term effects of calorie restriction *per se versus* weight loss *per se* on glucose and lipid metabolism, both at the whole-body and at the molecular level in obese patients with type 2 diabetes mellitus.

In this introduction, firstly the main actions of the "master" hormone in glucoregulation, insulin, will be discussed. Secondly, the normal regulation of blood glucose levels will be considered, both at the whole-body level as well as at the molecular level. Thirdly, the pathophysiology of type 2 diabetes mellitus is discussed, with specific focus on insulin resistance, both at the whole-body and the molecular level, and potential mechanisms of insulin resistance will be stressed. Fourthly, the reason and goals of therapeutic interventions will be attended, along with possible therapies. Fifthly, our research aims will be formulated and the outline of this thesis will be presented.

1.2. INSULIN

1.2.1 Hormone production

Insulin is a hormone produced by the β-cells of the Islets of Langerhans in the pancreas. At birth about $3x10^{-5}$ islets are present, increasing to $1x10^{-6}$ islets during the first years of life. The islets contain various cell types which each produce different hormones. The β-cell produces insulin. Other important hormones are somatostatin, produced in the δ-cell, and glucagon, produced in the α-cell. The latter counteracts the effect of insulin in many ways. The β-cell is situated central in the islet of Langerhans whereas the other cells are located peripherally.

The human insulin gene is located on the short arm of chromosome 11. *Via* DNA/RNA re-synthesis, a precursor molecule known as pre-pro-insulin (98 amino acids, molecular weight [MW] 11.500) is produced in the endoplasmatic reticulum of the pancreatic β-cells. It is cleaved to proinsulin (86 amino acids, MW approximately 9000) directly after the molecule has left the ribosome. The proinsulin is transported to the Golgi apparatus, where packaging into clathrin-coated secretory granules takes place. Maturation of the secretory granule is associated with the loss of the clathrin coating. In addition, the proinsulin is converted into insulin and C-peptide (MW 3000) by proteolytic cleavage at two sites. Normal granules shed insulin and C-peptide in equimolar amounts, along with some proinsulin and so-called split-products (only partially cleaved proinsulin). Insulin (MW 5808) itself consists of an A-chain of 21 amino acids and a B-chain of 30 amino acids, which are connected by two disulfide bonds. The secreted insulin first passes the liver where a proportion of insulin is cleared *via* a receptor-mediated process after exerting its action[59-61] The proportion of insulin cleared during first-pass through the liver has been estimated to be about 50% in dogs[60] and approximately 40 to 80% in humans[62-65]. The plasma half-life time ($t_{½}$) of insulin is only 5-10 minutes. C-peptide, the 31 amino acid residue, has no known biological function. Since C-peptide is produced in equimolar amounts with insulin it can be used as a marker for insulin secretory capacity, because it is not cleared by the liver but by the kidney and has a longer $t_{½}$ than insulin[66,67].

1.2.2. Hormone secretion

The main trigger for insulin release is an increase in the plasma glucose concentration in the portal circulation. Plasma glucose is sensed and taken up by the β-cell *via* facilitated diffusion by the specific glucose transporter (GLUT)-2. Subsequently, glucose is metabolised by the cell, which sets free energy in the form of adenosine tri-phosphate (ATP). The increase in intracellular ATP induces a closure of the ATP-dependent potassium channel at the cell membrane of the β-cell. This causes a depolarisation of the cell membrane, which leads to an opening of the voltage-dependent calcium channels and an inflow of calcium ions into the cell. The increase in intracellular calcium concentration eventually leads to the release of insulin from the granulae *via* exocytosis (Fig. 1)[66,67]

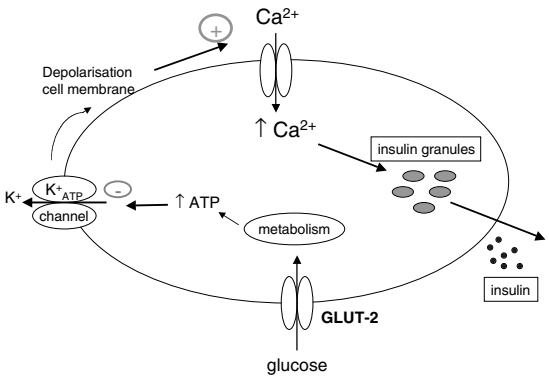

Figure 1.
See text for explanation (section 1.2.2 insulin secretion, page 18).

Several phases of insulin secretion can be identified: (i) basal insulin secretion is the way insulin is released in the post-absorptive state; (ii) the cephalic phase of insulin secretion is evoked by the sight, smell, and taste of food (before any nutrient is absorbed by the gut), and is mediated by pancreatic innervation; (iii) first-phase insulin secretion is defined as the initial burst of insulin, which is released in the first 5–10 min after the β-cells are exposed to a rapid increase in glucose (or other secretagogues); (iv) after the acute response, there is a second-phase insulin secretion, which rises more gradually and is directly related to the degree and duration of the stimulus; (v) finally, a third phase of insulin secretion has been described, albeit only *in vitro*. During all these stages, like many other hormones, insulin is secreted in a pulsatile fashion, resulting in oscillatory concentrations in peripheral blood. Oscillations include rapid pulses (recurring every 8-15 min) superimposed on slower, ultradian oscillations (recurring every 80-120 min) that are closely related to fluctuations in the glucose concentration[68-71]. This pulsatile pattern of insulin delivery to the liver is regulated mainly by modulation of insulin pulse mass in response to stimuli. The mass of insulin pulses through the liver is the predominant determinant of hepatic insulin clearance[65].

Table 4. Metabolic actions of insulin at the whole-body level.

	Stimulation of	**Inhibition of**
Liver	glycogen synthesis	gluconeogenesis
	protein synthesis	glycogenolysis
	lipogenesis	ketogenesis
Muscle	glucose transport	
	glycogen synthesis	
	protein synthesis	proteolysis
Adipose tissue	glucose transport	
	lipogenesis	lipolysis

1.2.3 Hormone action

Insulin is an anabolic hormone, which means that insulin facilitates the storage of energy sources, such as fat and glycogen, and stimulates protein synthesis. Because, physiologically, insulin is secreted following energy intake, insulin not only directs these energy sources towards storage, but simultaneously prevents endogenous release of energy sources (free fatty acids through lipolysis, proteolysis, *de novo* glucose production by the liver and ketogenesis), because these substrates are redundant in times of plenty. The effects of insulin on the various tissues are depicted in Table 4[66,67].

1.3 NORMAL GLUCOSE REGULATION

1.3.1. Glucose homeostasis at the whole-body level

Blood glucose levels are usually tightly regulated between 4-8 mmol/L. Low blood glucose levels are dangerous because brain function depends on glucose, and lack of glucose in the brain can cause seizures, loss off consciousness and death. On the other hand, elevated blood glucose levels can lead to either ketoacidosis or hyperglycaemic hyperosmolar dehydration in the acute situation, which can both eventually result in a coma. Furthermore, prolonged elevation of blood glucose levels can result in micro- (retinopathy, nefropathy, neuropathy) and macrovascular long-term complications.

The tight regulation of plasma glucose levels is achieved by the finely tuned hormonal regulation of glucose uptake by the tissues (rate of disappearance, R_d) on the one hand and glucose production on the other hand (rate of appearance, R_a)[72].

Glucose uptake by peripheral tissues is either insulin-independent (in the brain) or insulin-dependent (in muscle and adipose tissue). The brain cannot store glucose and, as mentioned before, is critically dependent on glucose for its function. Therefore, in the non-fed (= post-absorptive) state a certain level of endogenous glucose production is necessary. Glucose appearing in the post-absorptive state is mainly derived from the liver[73], although the kidney is also capable of glucose production. The amount of glucose produced by the kidney has been reported to be less than 5% after an overnight fast to 20% after a 60-h fast[73]. However, higher estimates of the contribution of the kidney to total post-absorptive gluconeogenesis have been reported. These differences depend on the techniques used to quantify renal glucose production. A significant role for the kidney in carbohydrate metabolism in type 2 diabetes has recently been proposed[74,75]. In healthy individuals the amount of endogenous glucose production (EGP, both liver and kidney) in the post-absorptive state averages 1.8-2.3 $mg.kg^{-1}.min^{-1}$ [73,76-78], which is about 10.0-12.8 $\mu mol.kg^{-1}.min^{-1}$.

Endogenous glucose production comprises 2 pathways: glycogenolysis, which is the breakdown of glucose stored as glycogen, and gluconeogenesis, which is the synthesis of new glucose molecules from precursor molecules like amino acids (mainly alanine), glycerol and lactate.

Endogenous glucose production is mainly regulated by fluctuations in the insulin/glucagon ratio in the portal vein[79,80]. Following a meal, insulin secretion is stimulated and the increase in portal vein insulin concentration inhibits endogenous glucose production *via* inhibition of glycogenolysis and gluconeogenesis. When the meal has been absorbed, plasma glucose levels decrease, even to a level a little below normal post-absorptive levels. This relative hypoglycaemia leads to increased secretion of glucagon. The subsequent elevation in portal vein glucagon concentration stimulates glycogenolysis and hepatic glucose production[81]. Endogenous glucose production is also influenced by other hormones (cortisol, growth hormone), free fatty acids (FFA), gluconeogenic precursors, paracrine substances (cytokines, prostaglandins) and the autonomic nervous system. All these factors keep endogenous glucose production relatively constant, a process called hepatic autoregulation[82-84].

Insulin-stimulated glucose uptake primarily takes place in skeletal muscle and amounts about 0.5 $mg.kg^{-1}.min^{-1}$ (the remainder of the average basal glucose uptake of 2.0-2.2 $mg.kg^{-1}.min^{-1}$ being utilised by the brain [1.0-1.2 $mg.kg^{-1}.min^{-1}$] and red blood cells)[85,86]. Glucose taken up in the muscle can either be oxidised to pyruvate (aerobic glycolysis) or lactate (anaerobic glycolysis) or stored as glycogen (non-oxidative glucose metabolism). Insulin-stimulated glucose oxidation seems to be bound to a maximum, making non-oxidative glucose disposal quantitatively the most important[87].

Of the three, for diabetes mellitus pathogenetically important, insulin-sensitive tissues, adipose tissue is the most sensitive for insulin. The EC_{50} value (i.e., the molar concentration of insulin that produces 50% of the maximum possible response that insulin is capable of) for suppression of lipolysis by insulin is between 7 and 16 μU/mL[76,88-92], whereas the EC_{50} values

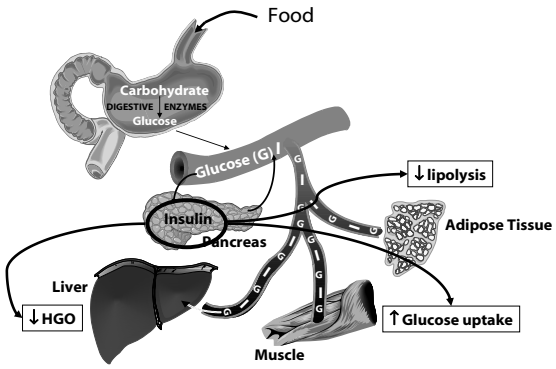

Figure 2.

The sight, smell and taste of food already stimulate insulin secretion. However, the rise of serum glucose levels following the consumption of a meal elicits a much more pronounced response (see text on page 19). Subsequently, insulin suppresses endogenous glucose production and lipolysis and stimulates whole-body glucose uptake. The duration of the increased insulin secretion following a meal is related to the degree and duration of hyperglycaemia.

for suppression of EGP of the liver and stimulation of glucose uptake in skeletal muscle, in normal subjects, are 26 μU/mL and 58 μU/mL, respectively[93].

The differences in the insulin dose-response curve between the various tissues are necessary for normal glucose and lipid metabolism. During an overnight fast, serum insulin levels are sufficiently low as to not to inhibit lipolysis (which provides free fatty acids and hence ketone bodies for the brain and glycerol for gluconeogenesis) and endogenous glucose production (providing glucose for the brain), but, on the other hand, are not high enough for maximum stimulation of (skeletal muscle) glucose uptake. After a meal, serum insulin levels rise, which stimulates glucose uptake and inhibits lipolysis and glucose production. The latter is achieved directly, by inhibition of gluconeogenesis and glycogenolysis, as well as indirectly, *via* inhibition of lipolysis, which diminishes the supply of glycerol and free fatty acids to the liver[66,67]. Fig. 2 shows what happens when a meal has been consumed.

1.3.2. Insulin signalling, molecular mechanisms regulating glucose uptake

Glucose transport and metabolism, protein synthesis and gene expression are all regulated by activation of the insulin-signalling pathway. Insulin signalling aimed at increasing the rate of glucose transport will be discussed below.

Glucose cannot pass the lipid bilayers of the cell membrane and needs a transporter to enter the cell. GLUT-4 is the main insulin-responsive glucose transporter and is located primarily in skeletal muscle cells and adipocytes. In unstimulated fat or muscle cells, 3-10% of GLUT-4 is located at the cell surface and more than 90% is located inside the cell in distinct vesicles[94]. In response to insulin, exercise and contraction, GLUT-4- containing vesicles move to and fuse with the plasma membrane, thereby increasing the number of GLUT-4 molecules in the membrane and, hence, increasing the rate of glucose transport into the cell[94]. Insulin elevates the exocytic rate of GLUT-4 and reduces its endocytotic rate only minimally. A review[95] on the different intracellular compartments containing GLUT-4 and the proteins that form the cytoskeleton along which GLUT-4 travels is beyond the scope of this thesis; it has not been investigated here.

Insulin is an important mediator of insulin-stimulated glucose transport that begins with binding of insulin at its receptor leading to a signalling cascade that eventually leads to the translocation of GLUT-4 to the cell membrane.

The heterotetrameric insulin receptor consists of 2 extracellular, ligand binding α-subunits and 2 transmembrane β-subunits containing tyrosine kinase domains[96,97]. When insulin binds to specific regions of the α-subunit, a rapid conformational change results in phosphorylation of the intracellular tyrosine residues on one half of the receptor dimer by the kinase domain of the other half, a process called autophosphorylation[98-100]. The phosphotyrosines on the insulin receptor can now serve as docking sites for phosphotyrosine binding (PTB)-domains on other proteins, such as insulin receptor substrates (IRS-1 to 4), Shc and Gab-1[101].

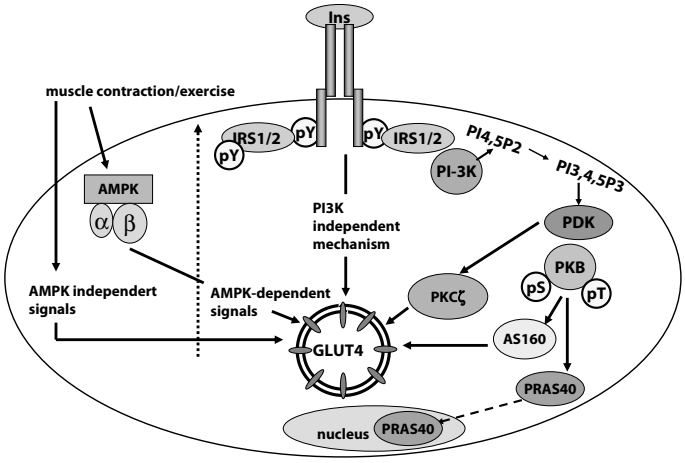

Figure 3.

Binding of insulin at the insulin receptor leads to phosphorylation of the receptor and insulin receptor substrates (IRS). Activated IRS-1 and -2 form a complex with phosphatidylinositol 3-kinase (PI3K) and this IRS/PI3K complex subsequently catalyses the formation of 3'-phosphoinositides (phosphatidyl-inositol-3,4-biphosphate [PIP2] and phosphatidyl-inositol-3,4,5-triphosphate [PI3P]). PIP3 attracts phosphoinositide-dependent kinase-1 (PDK-1) to the cell membrane and the complex subsequently activates protein kinase C (PKC) or protein kinase B (PKB/Akt), which are both involved in GLUT-4 trafficking to the cell membrane. The PKB/Akt substrate AS160 has recently been discovered as an intermediate in this process. Insulin-independent pathways involved in GLUT-4 translocation involve adenosine monophosphate-activated kinase (AMPK)-dependent (contraction, hypoxia) and -independent pathways.

IRS-1 and -2 appear to be the important mediators of insulin signalling in humans. IRS-1 is specifically involved in skeletal muscle and IRS-2 in adipose tissue insulin signalling[102]. Tyrosine phosphorylated IRS recruits and activates signalling molecules with src2-homology (SH2) domains, including phosphatidylinositol 3-kinase (PI3K)[103].

The IRS-PI3K complex catalyses the formation of 3'-phosphoinositides (phosphatidyl-inositol-3,4-biphosphate [PIP2] and phosphatidyl-inositol-3,4,5-triphosphate [PI3P]). PI3P serves as an allosteric regulator of phosphoinositide-dependent kinase (PDK), attracting PDK-1 to the cell membrane. There, PDK-1 activates (by phosphorylation) downstream mediators, such as protein kinase B (PKB/Akt) and atypical protein kinase C (aPKC, PKCζ/λ).

PKB/Akt is a serine/threonine kinase with 3 different isoforms, Akt 1, 2 and 3. Akt 2 is essential for normal glucose homeostasis[104,105]. After co-localisation with PDK-1[106], PKB/Akt is activated by phosphorylation of its two principal regulatory sites, Thr308 and Ser473[107]. Phosphorylation of both sites is essential for activation of PKB/Akt. Following activation, PKB/Akt dissociates from the cell membrane to affect metabolic processes[108,109]. Parts of the activated PKB/Akt also translocate to the nucleus to affect gene expression (see Fig. 3). The metabolic processes affected by PKB/Akt are glucose transport (*via* a stimulatory effect on GLUT-4 translocation) and glycogen synthesis. By inactivating glycogen synthase kinase-3 (GSK-3) the inhibitory action of GSK-3 on glycogen synthase[110] is abrogated and glycogen synthesis is stimulated[111].

With respect to the stimulatory effect of activated PKB/Akt on the translocation of GLUT-4 to the cell membrane, numerous studies have linked PKB/Akt to the regulation of glucose metabolism but the endogenous substrates regulating these responses are only beginning to be identified. Recent evidence suggests that the protein Akt substrate of 160 kDa (AS160) is involved as an intermediary in this process. AS160 is a protein containing a GTPase-activating domain (GAP) for Rab proteins, which are small G-proteins required for membrane trafficking[112,113]. Phosphorylation of AS160 is required for the insulin-induced translocation of GLUT4 to the plasma membrane in 3T3-L1 adipocytes[114]. Another recently discovered PKB/Akt substrate, proline-rich Akt-substrate 40 (PRAS40, also known as Akt1 substrate 1(Akt1S1))[115,116], is ubiquitously expressed and appears to be localised in the nucleus[116,117]. In response to growth factors, PRAS40 is phosphorylated on Thr246 *via* a PI3K- and PKB/Akt-dependent mechanism[115,117]. Phosphorylation of PRAS40 facilitates the binding of 14-3-3-proteins *in vitro*, and this protein complex has been implicated in nerve growth factor (NGF) mediated neuroprotection from ischaemia[117]. Although, PRAS40 is phosphorylated in response to insulin-treatment of cultured cell lines[115,118], it is as yet unknown whether this protein is involved in physiological insulin action.

As mentioned earlier, GLUT-4 translocation and, hence, glucose uptake can also be mediated *via* insulin-independent pathways, involving AMP-activated protein kinase (AMPK)[119] and other intermediates[120]. Interestingly, AS160 contains motifs similar to sequences of proteins that are phosphorylated by protein kinase C (PKC)[121] and AMPK[122]. In fact, muscle contraction phosphorylated AMPK, Akt and AS160 in isolated rodent muscle and chemical activation of AS160 caused AS160 phosphorylation[123]. Possibly, AS160 may act as a common effector of insulin and exercise signalling to recruit GLUT-4 to the plasma membrane.

Another PDK-1 substrate (*via* PI3-kinase) is atypical protein kinase C. In the liver aPKC regulates the expression of sterol regulatory element binding protein-1c (SREBP-1c), a transcription factor that activates numerous genes, including fatty acid synthase (FAS) and acetyl-coenzyme A carboxylase, that control lipid synthesis in the liver[124].

The insulin signal also has to be terminated in order to maintain metabolic control; this is established *via* specific phosphatases. Protein tyrosine phosphatase-1B (PTP1B) is a physiologic negative inhibitor of insulin signalling. By dephoshorylating the activated insulin receptor it terminates the insulin signal transduction[125]. In addition, SH2-domain-containing inositol phosphatases SHIP1 and SHIP 2 terminate PI3K signalling *via* dephosphorylation of the 5-position of the inositol ring of PIP3, to produce $PI(3,4)P_2$. The phosphatase PTEN (phosphatase and tensin homologue) dephosphorylates the 3-position on PIP3, producing $PI(4,5)P_2$ [126]. All three phosphatases can be regarded as potential therapeutic targets for type 2 diabetes mellitus.

1.4. TYPE 2 DIABETES MELLITUS

Type 2 diabetes mellitus is a chronic, multifactorial disease characterised by a combination of impaired insulin secretion by the pancreatic β-cells and insulin resistance of target organs, leading to hyperglycaemia. A diagnosis of diabetes mellitus is made when at least one of these three criteria is met: (i) symptoms of diabetes (polyuria, polydipsia, unexplained weight loss) with a casual blood glucose concentration > 11.1 mmol/L, (ii) fasting plasma glucose (FPG) level over 7.0 mmol/L, (iii) 2-h plasma glucose level > 11.1 mmol/L during an oral glucose tolerance test (OGTT)[127,128]. If no symptoms are present, one of these criteria must be present on a subsequent day.

Both conditions, i.e., deficient insulin secretion and insulin resistance, are necessary for diabetes mellitus to occur. Insulin resistance and a disturbed first-phase insulin response occur at an early stage in the development of type 2 diabetes mellitus. There seems to be a continuum from normal glucose tolerance to diabetes mellitus. Insulin resistance leads to increased insulin secretion by the pancreatic β-cell. This increase in insulin secretion is sufficient to offset hepatic insulin resistance (thereby maintaining a normal rate of basal hepatic glucose production) and to overcome the defect in muscle glucose uptake. At this moment, normal glucose levels are achieved at the expense of elevated serum insulin levels. In the second phase, the β-cells fail to compensate for the insulin resistance during glucose loads (as occurs during meals), leading to a condition known as impaired glucose tolerance (IGT). The cause is a disturbed first-phase insulin response, which normally suppresses endogenous glucose production. Over the years, the β-cell function deteriorates and when insulin secretion is no longer able to compensate for the insulin resistance hyperglycaemia ensues and a diagnosis of type 2 diabetes mellitus is made[22,129,130]. The relation between insulin secretion and insulin sensitivity is shown in Fig. 4 and the time-course of type 2 diabetes mellitus in Fig. 5.

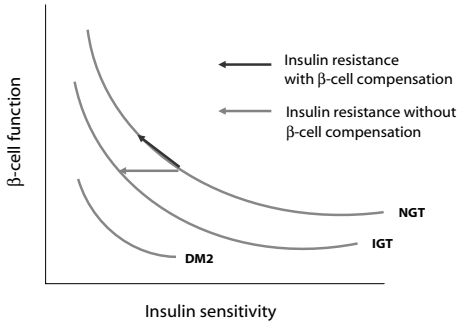

Figure 4.
In people with normal glucose tolerance (NGT), the relation between insulin sensitivity and β-cell function is curvilinear. See text for explanation (page 25).

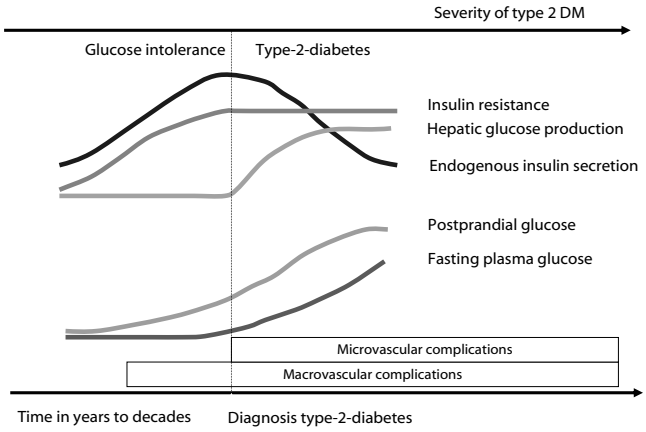

Figure 5.
Time course of type 2 diabetes mellitus. See text (page 25) for explanation.

1.4.1. Insulin resistance at the whole-body level

Insulin resistance at target organs leads to decreased glucose uptake, increased glucose production and increased whole-body lipolysis. Therefore, in patients with type 2 diabetes mellitus, basal glucose production is significantly elevated, leading to fasting hyperglycaemia. In addition, following a meal, insulin resistance leads to inadequate stimulation of (skeletal muscle) glucose uptake and insufficient suppression of endogenous glucose production and lipolysis. The result is postprandial hyperglycaemia.

The incapability to suppress whole-body lipolysis substantially contributes to the increased endogenous glucose production and diminished glucose uptake. Firstly, NEFAs increase endogenous glucose production by stimulating key enzymes involved in gluconeogenesis and by providing the energy needed for glucose production[22]. Secondly, the glycerol formed by triglyceride hydrolysis serves as a gluconeogenic substrate. Thirdly, free fatty acids impair insulin stimulated glucose uptake. Besides substrate competition (Randle effect)[131], impairment of insulin signalling appears to be responsible for this effect[132] (see next section).

1.4.2 Molecular mechanisms of insulin resistance

Skeletal muscle

Over 80% of insulin-stimulated glucose disposal takes place in skeletal muscle[86]. The main defect in patients with type 2 diabetes mellitus seems to reside in non-oxidative glucose disposal (NOGD), i.e., glycogen synthesis[133], the major pathway for overall glucose metabolism. With increasing obesity and insulin resistance, insulin-stimulated NOGD becomes more

impaired[134,135]. In patients with overt diabetes mellitus, the rate of glycogen formation was 60% that of normal subjects[133].

Possible mechanisms involved in decreased glycogen synthesis could either be decreased hexokinase activity, diminished glycogen synthase activity or impaired GLUT-4 translocation. Shulman *et al*. using ^{31}P-and ^{13}-C-nuclear magnetic resonance (NMR) spectroscopy showed that the defects were not at the level of hexokinase[136] or glycogen synthase[137] activity, but that impaired glucose transport appears to be the prime defect in insulin-stimulated glycogen synthesis in type 2 diabetic patients. The defects in glucose transport can either be due to defects in the glucose transporter itself or in translocation of GLUT-4 to the cell membrane.

Polymorphisms of the gene encoding GLUT-4 are very rare[138-140] in patients with type 2 diabetes and have the same prevalence in non-diabetic subjects. In addition, GLUT-4 protein and mRNA expression are equal[141,142] or even higher[143] as compared with normal subjects. However, GLUT-4 does have an abnormal subcellular distribution in insulin-resistant subjects with or without diabetes[144]. This indicates that translocation of GLUT-4 from intracellular compartments to the plasma membrane is the prime defect. Hence, defects in the signalling cascade leading to GLUT-4 translocation have been extensively investigated. It appeared that exercise (i.e., non-insulin dependent)-induced GLUT-4 translocation is normal in type 2 diabetic patients[145], but that insulin-stimulated GLUT-4 translocation is impaired[146]. Several defects in the insulin-signalling pathway have already been found and will be discussed below.

Insulin binding at the insulin receptor and protein expression of the insulin receptor are normal in skeletal muscle of patients with type 2 diabetes[147-149]. Both impaired[147,150,151] and normal[149,152,153] receptor tyrosine kinase phosphorylation and/or activity have been reported in subjects with diabetes. However, it is widely believed that the disturbance in GLUT-4 translocation in type 2 diabetes mellitus is due to a post-receptor defect.

IRS-1 is the first molecule downstream in the insulin-signalling cascade and plays a key role in skeletal muscle insulin signalling. In humans, IRS-1 polymorphisms are significantly more common in type 2 diabetic patients than in controls[154,155], but their role in the development of insulin resistance and type 2 diabetes is unclear[103]. Furthermore, in obese insulin- resistant subjects[156,157] and moderately overweight type 2 diabetic patients[149,156,158-160], insulin-stimulated IRS-1 phosphorylation in skeletal muscle is decreased as compared to control subjects, whereas protein expression is not altered[149,156,159]. This defect can already be found in normoglycaemic relatives of type 2 diabetic patients[161]. The cause seems to be serine/threonine phosphorylation of IRS-1, which thereby loses its ability to act as a substrate for the tyrosine kinase activity of the insulin receptor and inhibits its capacity to bind to and activate downstream effector molecules such as PI3K[162,163]. Here, a link with adipocyte biology (and obesity) can be made, since circulating FFAs and TNF-α have been found to increase serine phosphorylation of IRS-1[132].

PI3-kinase is central in the insulin-signalling cascade; however, its activation is necessary but not sufficient for the metabolic actions of insulin. A common polymorphism of the p85-α subunit of PI3K (Met326Ile) was found in two percent of a Caucasian study population in homozygous form, leading to a 32% reduction in insulin sensitivity during an intravenous glucose tolerance test as compared to wild type and heterozygous carriers. The frequency of the polymorphism is not increased in diabetes however[164], but insulin-stimulated PI3K activity is impaired in obese subjects [156], as well as in moderately overweight type 2 diabetic patients[156,158,159,165].

Little is known about the physiological regulation of PDK-1, but thus far insulin action on PDK-1 appears to be normal in insulin-resistant skeletal muscle[158]. With respect to PKB/Akt, unravelling its role in insulin resistance has been complicated by the existence of three isoforms. It appears that Akt 2 is essential in glucose homeostasis, Akt 2 knockout mice having insulin resistance and a diabetes mellitus-like syndrome[104]. In humans, recent studies have detected a missense mutation in the kinase domain of PKB-β (Akt2) in a family of severely insulin-resistant patients that was preserved over three generations[166]. Not only was the mutant Akt unable to phosphorylate downstream effectors in the insulin-signalling pathway, but it also inhibited phosphoenolpyruvate carboxykinase (PEPCK), a gluconeogenic enzyme. In humans with type 2 diabetes mellitus, basal PKB/Akt activity was similar to controls. Two *in vivo* studies showed normal insulin-stimulated activation of PKB/Akt[165,167] in patients with type 2 diabetes mellitus, although one study used supraphysiological concentrations of insulin[165]. In contrast, *in vitro* experiments showed decreased insulin-stimulated PKB/Akt activity at high levels and normal activity at low insulin levels[168] in human muscle strips of type 2 diabetic patients. The defect seems to be isoform specific[169] and a defect in one isoform might be masked by increased activity of the other.

With respect to the recently discovered Akt substrate AS160, Karlsson *et al*. showed that AS160 phosphorylation is impaired in skeletal muscle from type 2 diabetic patients[170].

Liver

Insulin signalling in the liver differs from that in skeletal muscle (and adipose tissue). In muscle, IRS-1 (*via* PI3K) controls both activation of aPKC and PKB/Akt, whereas in the liver aPKC is controlled (again *via* PI3K) by IRS-2 and PKB/Akt by IRS-1. In muscle and adipocytes, aPKC and PKB/Akt stimulate the transportation of GLUT-4 to the cell membrane. In the liver, aPKC regulates the expression of SREBP-1c, a transcription factor that activates numerous genes, including FAS and acetyl-coenzyme A carboxylase, that control lipid synthesis in the liver. PKB/Akt in the liver is involved in the control of glucose production[124].

When insulin activates PKB/Akt (*via* IRS-1), this results in the phosphorylation of Foxo-family transcription factors (Foxo-1a,-3a and -4). These Foxo-transcription factors can bind to so-called insulin response elements (IRE) on the promotor regions of (among others) two key gluconeogenic enzymes: PEPCK and the glucose-6-phosphatase catalytic subunit (G6Pase),

thereby inhibiting their expression[171,172]. Defective IRS-1 signalling to PKB/Akt leads to lack of inhibition of these two enzymes and increased glucose production[124,173].

IRS-2-mediated signalling to aPKC in the liver of diabetic rodents is largely intact or elevated. This might explain the increased very-low-density lipoprotein (VLDL)-triglyceride synthesis in type 2 diabetes[124].

Hepatocyte nuclear factor (HNF) may also play a role in insulin-mediated glucose metabolism in the liver. HNF-1 enhances the effect of insulin on the promoter of the gene encoding G6Pase *via* interaction with an IRE[174]. Knockout mice that are homozygous for a null mutation in the HNF-3 gene have a complex impairment of glucose metabolism with persistent hypoglycaemia[175]. Finally, HNF-4 is involved in the PI3K/PKB/Akt-dependent stimulation of glucokinase gene expression by insulin, a mechanism involved in increasing glycolysis[176]. On the molecular level HNF-4 seems to interact with Foxo-1[177]. However, although genetic defects of some of the HNF transcription factors play a role in some forms of maturity-onset diabetes of the young (MODY), thus far no evidence exists that HNF-transcription factors are involved in type 2 diabetes mellitus.

GSK-3, an enzyme regulating glycogen synthesis, is a substrate of PKB/Akt. Normally, GSK-3 is constitutively active, phosphorylating glycogen synthase (GS), which becomes inactive and thus glycogen synthesis is inhibited. Insulin promotes glycogen synthesis *via* PKB-mediated inhibition of GSK-3. Defective glycogen synthesis is not only evident in skeletal muscle of patients with insulin resistance but also in the liver. Polymorphisms in the glycogen synthase gene have been described in insulin-resistant patients, the most frequent being the XbaI and Met416Val mutations within intron 14 and exon 10, respectively[178].

In conclusion, in the liver impaired insulin signalling from IRS-1 to PKB/Akt leads to increased glucose production *via* inhibition of gluconeogenic enzymes. In addition, glycogen synthesis is inhibited and, at least in rodents, impaired IRS-2 signalling to aPKC leads to increased VLDL synthesis. Unfortunately, ethical considerations do not permit liver biopsies in humans to study the pathogenetic abnormalities in patients with type 2 diabetes mellitus.

Adipose tissue

About 10% of whole-body glucose uptake occurs in adipose tissue. This might suggest that adipose tissue is of minor importance in insulin-stimulated glucose disposal and in insulin resistance. However, in mice, adipose-tissue-specific GLUT-4 knockout leads to a similar degree of insulin resistance in muscle and liver as muscle-specific GLUT-4 ablation[179,180]. In addition, muscle GLUT-4 depletion is associated with a markedly enhanced glucose uptake in adipose tissue[181]. Hence, there seems to be cross-talk between adipose tissue and skeletal muscle, and adipose tissue seems to be of major importance in the development of insulin resistance. This will be discussed in Chapter 2.

Insulin-stimulated glucose uptake in adipose tissue takes place *via* the same mechanism as in skeletal muscle: insulin signalling leading to GLUT-4 translocation. However, discrepan-

cies have been found as to the defects in the insulin-signalling cascade in type 2 diabetic patients, between adipose tissue and skeletal muscle cells. In adipose tissue defects are related to decreased protein expression, whereas this is normal in skeletal muscle. Hence, IRS-1 phosphorylation in adipose tissue of patients with type 2 diabetes is decreased because of a decreased IRS-1 protein expression (by 70%) and PI3K activity is decreased to the same extent by decreased protein expression[182]. In addition, in adipose tissue IRS-2 is capable to compensate for changes in IRS-1[182], a phenomenon that does not seem to occur in skeletal muscle[149].

PKB/Akt activation is also impaired in adipose tissue of type 2 diabetic subjects, primarily *via* a reduction in insulin-stimulated serine phosphorylation[183]. GLUT-4 protein and mRNA expression are also substantially reduced in adipose tissue of type 2 diabetic patients[184], in contrast to the normal expression in skeletal muscle[141,142,185].

The main interest in the role of adipose tissue in whole-body insulin resistance has been on so called adipocytokines (or even better, adipokines, since not all proteins secreted by adipocytes are cytokines), proteins secreted by the adipocyte that might induce insulin resistance. This will be discussed shortly below and more extensively in Chapter 2.

1.4.3 How are changes in skeletal muscle insulin resistance induced?

Both FFAs and several adipokines derived from adipose tissue can influence insulin sensitivity.

It has been recognised for some time that insulin sensitivity is inversely related to fasting plasma FFA levels[186-188]. Furthermore, a strong inverse relationship has been demonstrated between intramyocellular lipid (IMCL) levels and skeletal muscle insulin sensitivity[189-192]. Endurance-trained athletes also have high levels of IMCLs, but they have a high insulin sensitivity[193]. It seems that the capacity to oxidise these IMCL is of prime importance in inducing insulin resistance. This has also been called metabolic flexibility[194,195]. It appears that metabolically-flexible persons (lean, aerobically fit, healthy individuals) have a preference for fat oxidation in muscle during fasting and that during insulin stimulation this fat oxidation is suppressed and glucose oxidation is stimulated[196]. In metabolically-inflexible people there is both a blunted preference for fat oxidation in the fasted state and a blunted suppression of fat oxidation upon insulin stimulation[197-199]. Hence, athletes appear to have a high IMCL content because they prefer to oxidise fat, with the intramyocellular triglycerides (present in high concentration) serving as an energy reservoir. Whereas in obese and/or type 2 diabetic patients, elevated IMCL seem to be secondary to a structural imbalance between plasma FFA availability, fatty acid re-esterification and oxidation. The defect in fat oxidation seems to reside in the mitochondria[200].

Apart from defects in intracellular fatty acid oxidation and or re-esterification, another mechanism leading to increased IMCL might be *via* increased fatty acid uptake. Long-chain fatty acids (LCFA) enter cells mainly by protein-mediated membrane transport, along with

passive diffusional uptake[201]. One of these proteins is the fatty acid transporter (FAT)/CD36. FAT/CD 36, like GLUT-4, is usually located in the cytoplasm and can be acutely translocated to the sarcolemma by stimuli such as contraction and insulin[202-206]. Both in animal models[207] of insulin resistance, as well as in obese non-diabetic and non-obese diabetic humans[202], FAT/CD36 membrane expression was increased as compared to lean controls. Moreover, this increased sarcolemmal FAT/CD36 expression was associated with an increase in LCFA uptake[202,208]. In the human study, the increase in LCFA transport led to a 3-fold increase in fatty acid esterification, whereas fatty acid oxidation remained the same, again indicating that the core defect is in mitochondrial fatty acid oxidation[202].

Hence, any perturbation that leads to a defect in mitochondrial fatty acid oxidation (aging, potential type 2 diabetes genes) and/or increased delivery of fatty acids (increased caloric intake, obesity, increase in FAT/CD36) can lead to intramyocellular lipid accumulation.

ICML, in turn, can impair insulin signal transduction. It has been proposed that fatty acid metabolites induce a sustained activation of serine/threonine kinases, like protein kinase C isoforms[209-211], IκB kinase-β[212,213] and Jun N-terminal kinase[163,214], which phosphorylate IRS-1 and IRS-2 on serine and threonine sites. Serine-phosphorylated forms of IRS-1 and-2 cannot associate with and activate PI3K, resulting in a decreased activation of GLUT4-regulated glucose transport.

Another adipocyte product, TNF-α, also induces insulin resistance *via* serine/threonine phosphorylation of IRS-1, thereby inhibiting insulin signalling[215-217].

An extensive review of adipokines and their potential impact on insulin sensitivity is presented in Chapter 2.

1.4.4. Visceral obesity and insulin resistance

A chronic imbalance between energy intake and energy expenditure will eventually lead to obesity. Epidemiological studies have shown an association between severe obesity and increased mortality[20,218,219]. In more moderate obesity, regional distribution of fat seems to play an important role in the risk for (cardiovascular) morbidity and mortality[220-224]. As early as 1947 Vague put forward that "android or male-type obesity", is more often associated with increased mortality and risk for diabetes, hypertension, hyperlipidaemia and atherosclerosis than the "gynoid" (lower body or gluteofemoral) female-type of fat distribution[225]. Later, studies using imaging techniques (computer tomography [CT] and magnetic resonance imaging [MRI]) showed that the detrimental influence of abdominal obesity on metabolic processes is related to the intra-abdominal, i.e., visceral, fat depot and not to subcutaneous fat deposition[226-230]. However, other investigators have challenged a primary role for visceral adipose tissue in insulin resistance and showed that truncal subcutaneous adipose tissue is also strongly and inversely related to insulin-stimulated glucose disposal (reviewed by Garg *et al*.[231]). Moreover, given the fact that visceral adipose tissue contributes only 10-15% of the total systemic free fatty acid flux (the majority of FFAs being derived from non-splanchnic

adipose tissue from the rest of the body)[232,233], they questioned the impact of excess visceral adipose tissue on peripheral insulin sensitivity. However, liposuction of subcutaneous abdominal adipose tissue does not improve insulin sensitivity[152]. Moreover, although only 10-15% of fatty acids are derived from visceral adipose tissue, their drainage *via* the portal vein directly to the liver could imply another, more deleterious mechanism of action than delivery of FFAs (and adipokines) to the liver *via* the hepatic artery. Hence, it is not clear yet whether visceral adipose tissue is the culprit or whether the combination of truncal subcutaneous adipose tissue with visceral adipose tissue are involved in insulin resistance. Finally, it is also unclear whether abdominal obesity causes insulin resistance or is merely the reflection of the pathologic state.

Notwithstanding these uncertainties, available evidence does support an important role for adipose tissue in, possibly, generating and, at least, maintaining whole-body insulin resistance. Several theories have been put forward to explain the link between obesity and insulin resistance. The portal/visceral hypothesis[234] states that visceral fat cells are metabolically more active (especially lipolytic activity) and are less responsive to the antilipolytic effects of insulin as compared to other adipose tissue depots. Subsequently, the high flux of FFAs and glycerol derived from these visceral fat cells, through their unique drainage directly into the liver *via* the vena portae, would induce hepatic insulin resistance, increase hepatic glucose production and increase VLDL-triglyceride production. However, as mentioned in the previous paragraph, the portal/visceral hypothesis cannot link visceral adiposity to peripheral insulin resistance given the fact that only 10-15% of the total FFA flux is derived from visceral adipose tissue, unless some other factor released by visceral adipose tissue induces peripheral insulin resistance and/or visceral fat cells have impaired functioning in insulin-resistant states leading to decreased triglyceride storage and partitioning of fat storage into other organs. This is where 2 new theories emerge: (i) the adipocyte as an endocrine organ and (ii) the ectopic fat storage theory[235].

To begin with the first theory, adipose tissue not merely stores triglycerides but actively secretes lipid moieties such as FFAs and proteins that are called adipokines[236,237]. Quantitatively, FFAs are the most important. Moreover, elevated FFAs play a major role in inducing whole-body insulin resistance. Chronically elevated FFA levels stimulate hepatic glucose production and VLDL-triglyceride synthesis, leading to hyperglycaemia and dyslipidaemia[22]. Furthermore, chronically elevated FFA concentrations impair insulin signalling *via* serine/threonine phosphorylation of IRS-1, thereby decreasing insulin-stimulated glucose transport[132]. In addition, chronic exposure to high FFA levels to the pancreas can impair insulin secretion[238-240]. Several of the adipokines produced by adipose tissue (adiponectin, leptin, TNF-α) can also induce insulin resistance, this will be discussed in Chapter 2.

The theory of ectopic fat storage states that a diminished capacity of fat cells to store fat as triglycerides leads to lipid storage in other organs, such as the liver, pancreas and muscle (overflow hypothesis[241]/ectopic fat storage[235]). This causes steatosis hepatis with hepatic in-

sulin resistance, impaired insulin secretion and skeletal muscle insulin resistance (*via* IMCL and impaired insulin signalling, see previous section)[242]. The cause of ectopic fat storage is unclear but an association with enlarged adipocytes has been found[243]. This might be the result of impaired proliferation or differentiation of adipocytes. On the other hand, impaired whole-body fat oxidation might account for the ectopic accumulation of fat[244].

Hence, adipose tissue plays an important role in generating and maintaining insulin resistance *via* the excessive production of FFAs and insulin-resistance-provoking adipokines (TNF-α, IL-6, resistin, leptin and many others), possibly related to specific fat depots (visceral fat mass) and/or malfunctioning of adipocytes (in these specific depots?). Moreover, a diminished capacity to store fat leads to ectopic fat storage with lipotoxicity-induced impairments in function of insulin-responsive tissues such as the liver, muscle and pancreas.

1.5. OBESITY AND TYPE 2 DIABETES; TREATMENT REASONS, GOALS AND OPTIONS

Both obesity associated with insulin resistance (Table 1) and type 2 diabetes mellitus impose a major health risk. Patients with type 2 diabetes mellitus have an increased morbidity and mortality due to long-term micro- (retinopathy, neuropathy, nefropathy) and macrovascular complications. Patients with type 2 diabetes have a 2-4 fold increased relative risk (RR) for the development of myocardial infarction (MI), peripheral arterial disease and stroke[220] and approximately 65% of patients with type 2 diabetes die as a result of a cardiovascular event[245]. This increased risk is associated with chronic hyperglycaemia and an increase in cardiovascular risk factors such as hyperglycaemia, dyslipidaemia and hypertension. Hypertension occurs in up to 60% of patients with diabetes[246], and if diabetes and hypertension co-exist they exert a multiplicative effect on the absolute risk of a cardiovascular event[247]. Small dense LDL-cholesterol, high serum triglycerides and low HDL-cholesterol characterise diabetic dyslipidaemia. Hence, treatment of patients with type 2 diabetes should not only focus on glucoregulation but also on hypertension and dyslipidaemia.

Mainly based on two large prospective randomised studies investigating the effect of intensive blood glucose-lowering therapy on glycaemic control and occurrence of micro-and macrovascular complications in type 1 and type 2 diabetic patients[248,249], the treatment goals for glucoregulation in patients with type 2 diabetes as set by the ADA are: fasting plasma glucose level < 7.0 mmol/L, postprandial glucose level < 10 mmol/L and HbA_{1c} < 7%. In addition, systolic blood pressure should be lower than 130 mmHg and diastolic blood pressure under 80 mmHg. LDL-cholesterol should be < 2.6 mmol/L, triglycerides < 1.7 mmol/L and HDL-cholesterol > 1.1 mmol/L[250].

Theoretically, treatment of hyperglycaemia in patients with type 2 diabetes can consist of decreasing the need for insulin and/or increasing available insulin. The need for insulin

can be diminished either by decreasing postprandial glucose levels (diet, acarbose[251]) or improving insulin sensitivity. The latter can be achieved *via* restriction of caloric intake[252], weight loss[252], exercise[253], or with drugs: metformin[254,255] or thiazolidinediones[256,257] (perhaps also rimonabant[258] and sibutramine[259], because of their weight-loss-inducing properties, their anorexic effects and possibly *via* a direct beneficial effect on insulin sensitivity). Increasing available insulin can be achieved with insulin secretagogues (sulfonylureaderivatives[254], meglitinides[260]) or by giving exogenous insulin.

Weight loss improves multiple aspects of insulin resistance: glucoregulation, dyslipidaemia, hypertension and others. In addition, it decreases the risk for arthrosis, low back pain, gallstones, cancer, etc. So ideally, weight loss should always be a component of the treatment regimen in obese patients.

Weight loss also improves insulin resistance in obese non-diabetic patients. A beneficial effect of even 5-10% loss of overweight has been shown on dyslipidaemia, hypertension, hyperinsulinaemia and glucose values[261-263]. To date, no effect on incidence rates of myocardial infarction, stroke, cancer and mortality has been demonstrated, however.

Weight loss regimens have been proven difficult to adhere to. In addition, weight loss achieved through diet is often followed by weight regain. Regimens combining a hypocaloric diet (500 to 600 kCal less than needed per day) with behaviour therapy and exercise have been proven the most beneficial with respect to outcomes after 1 year[264]. However, hypocaloric diets often lead to only modest weight loss, whereas morbidly obese patients and obese type 2 diabetic patients need larger weight losses to restore peripheral insulin sensitivity[47,48]. VLCDs and bariatric surgery have been advocated for this purpose.

1.5.1 Bariatric surgery

Surgical procedures to treat obesity have been performed since the 1950s[265] and include truncal vagotomy[266], jaw wiring[267], intragastric balloons and liposuction. Bariatric (weight loss) surgery can be divided into purely restrictive procedures (vertical banded gastroplasty [VBG], laparoscopic adjustable silicone gastric banding [LASBG]) and combined restrictive and malabsorptive procedures (Roux-en-Y gastric bypass [GBP], biliopancreatic diversion [BPD])[57,268]. The latter induce larger weight losses and, hence, greater improvements in hypertension, dyslipidaemia, glucose metabolism and hyperinsulinaemia as compared to the purely restrictive techniques[50,56]. However, they are irreversible, sometimes leading to greater weight losses than necessary and also to nutritional deficiencies. Patients have to take vitamin supplements for the rest of their lives. LASBG is the most popular form of bariatric surgery in the Netherlands (and the rest of Europe), because it can be performed laparoscopic and therefore has fewer perioperative complications and it is reversible. In addition, some influence as to the amount of food intake can be exerted *via* inflation/deflation of the saline-filled gastric ring[57,268]. This procedure also has disadvantages however, an estimated 7-17% of the

patients has to be re-operated because of band erosion, dislocation or leakage or because of esophageal dilatation[269,270].

Bariatric surgery can induce large weight losses (20-50% of body weight) with a higher likelihood of maintaining weight loss (especially the combined restrictive and malabsorptive procedures) as compared to other weight loss interventions[55,271].

The Swedish Obese Subjects (SOS) study showed that surgically-treated obese subjects had about 25% percent greater weight loss at 10 years follow-up, along with a greater number of persons who no longer had diabetes (if present), hypertriglyceridaemia, low HDL-cholesterol concentrations, hypertension and hyperurikaemia as compared with conventionally treated obese subjects. The surgery group also had lower 2- and 10-year incidence rates of diabetes and hypertriglyceridaemia, but not hypercholesterolaemia[43]. Others have reported similar beneficial metabolic effects of bariatric surgery.

Bariatric surgery has also been performed in patients with type 2 diabetes. Although in some studies the number of patients with diabetes were small[52,55], the impressive results found were confirmed in larger studies[51,53]. A recent meta-analysis by Buchwald *et al*. showed that 1417 out of 1846 patients (76.8%) completely recovered from their diabetes following bariatric surgery (in the studies that mentioned complete resolution). The mean reduction in BMI was 14 kg/m^2 and a graded response with respect to diabetes resolution was noted with the greatest effect with BPD, whereas gastric banding was the least effective[56]. A recently published, retrospective chart review of 312 obese patients with type 2 diabetes that underwent biliopancreatic surgery (gastric bypass with biliopancreatic diversion), showed that the beneficial effects on glucose metabolism, dyslipidaemia and hypertension were maintained in most patients even after 10 years follow-up[53].

With respect to the underlying metabolic processes leading to the improvement in glucose metabolism following bariatric surgery, studies in morbidly obese patients have shown an improvement in insulin-stimulated glucose disposal, as assessed with the hyperinsulinaemic euglycaemic clamp technique[47,50,272,273]. Data on endogenous glucose production and whole-body lipolysis are not available. Moreover, in obese type 2 diabetic patients no studies using either of these sophisticated techniques have been performed to date.

1.5.2 Very low calorie diets

VLCDs typically provide less than 800 kCal/day. This can be achieved *via* adjustments of "normal" food intake or *via* commercially available packages. The advantage of the latter is that these products contain all the necessary vitamins, minerals and trace elements, so patients need not to figure out what to eat and what not.

VLCDs are safe[58] and can be used for several weeks to months or even up to one year([274] and own observations). VLCDs can also induce large weight losses[275]. Maintenance of weight loss is usually a problem, necessitating the need for regular dietary counselling and preferably also behaviour therapy.

Both in obese patients and in obese patients with type 2 diabetes mellitus, VLCDs lead to substantial weight loss and improvements in hyperglycaemia, hyperinsulinaemia, dyslipidaemia and hypertension[58,275-280].

In obese type 2 diabetic patients hyperglycaemia improves already within 4-10 days after the beginning of an energy restricted diet[277,278,281,282]. This appears to be due primarily *via* a decrease in endogenous glucose production. These studies have been performed when some (4-5 kg) weight loss had already occurred, with varying degrees of calorie restriction or in mild type 2 diabetic patients. Surprisingly, there are no studies documenting to what extent carbohydrate and lipid metabolism improve in obese, insulin-treated type 2 diabetic patients after substantial weight loss using a sophisticated method such as the hyperinsulinaemic euglycaemic clamp technique with [6,6-$^{2}H_{2}$]-glucose and [$^{2}H_{5}$]-glycerol.

1.6. AIMS OF THE STUDIES AND OUTLINE OF THE THESIS

Most patients with type 2 diabetes mellitus are obese and both obesity and type 2 diabetes mellitus are associated with insulin resistance. Therefore our **first aim** was to evaluate the role of adipose tissue (which indeed is present in excess in obese and obese diabetic patients) in insulin resistance. For this purpose we reviewed the literature and present a hypothesis which links adipose tissue to insulin resistance (**Chapter 2**).

In **Chapter 3**, we present an example of a hormone produced by adipose tissue (leptin) that is associated with insulin resistance. The relation between serum insulin and leptin is well established in obese patients and patients with diabetes, but not in very obese, largely insulin-treated patients with diabetes. Our **second aim** was to evaluate the relation between fasting serum leptin and fasting serum insulin levels, as well as between fasting serum leptin levels and insulin secretion in a group severely obese type 2 diabetic patients at various moments of energy restriction and weight loss.

Insulin resistance in very obese type 2 diabetic patients makes it often difficult to achieve adequate glycaemic regulation. Energy restriction and weight loss improve insulin resistance and its associated metabolic abnormalities. VLCDs can induce large weight losses but most type 2 diabetic patients are afraid to use these diets along with their blood glucose-lowering medication for fear of hyperglycaemia. Therefore, we wanted to stop all blood glucose-lowering agents at the start of the VLCD. This would also facilitate weight loss and enable us to study glucose metabolism without interfering medication. However, we did not want to induce severe hyperglycaemia or other metabolic derangements. Therefore, our **third aim** was to evaluate whether it is safe to treat very obese, insulin-treated type 2 diabetic patients with a VLCD (Modifast®, 450 kCal/day) and simultaneously discontinue all blood glucose-lowering medication, including insulin (**Chapter 4**).

Other studies mentioned a decline in blood glucose levels before weight loss occurred, even as early as 7 days after the initiation of a VLCD. Our own clinical observations suggested that blood glucose levels decrease already within 2 days after starting a VLCD. Because we wanted to differentiate later on between the effects of energy restriction *per se* and weight loss *per se* on glucose metabolism, our **fourth aim** was to establish whether blood glucose levels indeed decline as early as 2 days after the initiation of a VLCD and the discontinuation of all blood glucose-lowering agents, including insulin (**Chapter 4**).

Because we wanted to study the effect of calorie restriction and weight loss on lowering blood glucose levels, the patients entering our later studies should preferentially react to the VLCD with a decline in blood glucose levels. Therefore, our **fifth aim** was to find out whether there are discriminating factors that will tell in advance which patients will show a decline in blood glucose levels during weight loss with a VLCD and which patients will not (**Chapter 4**).

Subsequently, our **sixth aim** was to investigate, using the hyperinsulinaemic euglycaemic clamp technique with stable isotopes, at the whole-body level, the mechanisms by which calorie restriction *per se* (2-day VLCD) decreases blood glucose levels in obese insulin-treated type 2 diabetic patients in whom all blood glucose-lowering medication was discontinued at the start of the VLCD (**Chapter 5**). In this same study, our **seventh aim** was to unravel the blood glucose-lowering effect of a 2-day VLCD at the molecular level. To this end, we studied components of the insulin-signalling cascade, GLUT-4 and FAT-CD36 translocation and intramyocellular triglycerides in skeletal muscle biopsies taken on day 0 and day 2 of the diet, both in the basal as well as in the insulin-stimulated situation (**Chapter 6**).

In addition, our **eighth aim** was to differentiate between the effects of calorie restriction *per se* (day 2 of a VLCD) and those of weight loss *per se* (until 50% of overweight was lost), on whole-body glucose and lipid metabolism in obese insulin-treated type 2 diabetic patients in whom again all blood glucose-lowering medication was discontinued at the start of the VLCD (day 0) (**Chapter 7**). Our **ninth aim**, carried out in the same study, was to investigate whether calorie restriction *per se* and weight loss have differential effects on insulin signalling, GLUT-4 and FAT/CD36 translocation and the amount of intramyocellular triglycerides in skeletal muscle biopsies obtained on day 2 of a VLCD and again when 50% of overweight was lost, in the basal situation and during hyperinsulinaemia (**Chapter 8**).

Our **tenth aim** was to investigate whether the weight loss and beneficial metabolic effects of a once-only 30-day VLCD in obese type 2 diabetic patients, who were taken off all blood glucose-lowering therapy during that diet and who received standard outpatient care thereafter (blood glucose-lowering therapy was restarted if deemed necessary by their own doctor), were sustained at 18 months regular outpatient follow-up (**Chapter 9**).

In **chapter 10** the results of our studies are discussed and integrated.

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