Adiponectin in glucose metabolism

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1 General introduction
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1.1 Introduction

The world is currently experiencing a global epidemic of obesity. In the Netherlands ± 30% of adults are overweight (BMI 25-30 kg/m²) and ± 10% are obese (BMI>30 kg/m²). Among adolescents these rates are 20% and 3%, respectively 1. Obesity is a major risk factor for insulin resistance, type 2 diabetes mellitus and cardiovascular diseases.

For a long time adipose tissue has been viewed as a relatively inert storage depot for triglycerides. However, over the past years adipose tissue has been recognized as the largest endocrine organ in the body, synthesizing and secreting several biologically active hormones, the adipocytokines, that participate in the regulation of glucose and lipid metabolism. Among those adipocytokines is adiponectin, a relatively abundant plasma protein, which is produced and secreted predominantly by adipocytes 2. In animal experiments, administration of adiponectin ameliorates glucose metabolism by enhancing glucose uptake and suppressing hepatic glucose production 3-5. Plasma concentrations of adiponectin are low in insulin resistant patients with obesity, type 2 diabetes mellitus and HIV-associated lipodystrophy 6, 7. Considering the insulino-mimetic properties of adiponectin, the reduction of adiponectin could play a role in the pathogenesis and/or perseverance of insulin resistance in these patients. In addition, adiponectin could be involved in the etiology of the derangements in glucose metabolism found during sepsis or other acute infections.

This thesis examines the regulation of adiponectin and its role in the disturbances in glucose and lipid metabolism with an emphasis on patients with infections and HIV-lipodystrophy.

1.2 Glucose and lipid metabolism

1.2.1 Glucose metabolism

Maintenance of a constant blood glucose level is essential for normal physiology in humans. The plasma glucose concentration is the resultant of a balance between glucose supply and glucose utilization. Glucose can be derived from exogenous or endogenous sources. In fasting humans, glucose is predominantly produced by the liver and to a smaller extent by the kidney. The liver can produce glucose by breaking down glycogen (glycogenolysis) or by de novo glucose synthesis from gluconeogenic precursors (gluconeogenesis) such as lactate, glycerol and several amino acids (especially alanine and glutamine). After an overnight fast, the contribution of gluconeogenesis to hepatic glucose production
is approximately 50%, whereas after prolonged fasting this increases up to 95%. A prerequisite for glycogenolysis is the presence of sufficient glycogen stores. After an overnight fast liver glycogen is limited to 70-150 g. Total depletion of hepatic glycogen stores takes place within 64 hours of fasting. Therefore, during progressive starvation, the relative contribution of glycogenolysis to total glucose production decreases.

Glucose utilization forms the opposite side of the glucose balance. After peripheral uptake, glucose can be utilized via 2 separate pathways: oxidative and non-oxidative utilization. Glucose oxidation is the process of energy (ATP) synthesis from glucose molecules. Oxidation of glucose actually takes place in a complicated series of biochemical steps involving glycolysis, the Krebs cycle and the respiratory chain. From the oxidation of 1 glucose molecule, the net production of ATP molecules is 31. Non-oxidative glucose utilization mainly affects the storage of glucose as glycogen or fat. If large quantities of carbohydrates are consumed, exceeding immediate demands, the surplus of glucose is stored initially as glycogen and later on as fat. Glucose enters the pentose phosphate pathway and via the glycolytic route and the Krebs cycle, finally citrate is diverted into the cytosol for fatty acid synthesis. Three fatty acids are combined with glycerol-3-phosphate to form triacylglycerol (triglycerides).

1.2.2 Lipid metabolism
Adipocytes are able to synthesize triglycerides from glucose and FFA from exogenous sources. In addition to adipose tissue, the liver is also able to synthesize triglycerides, which are released into the circulation as very low density lipoproteins (VLDL) and exported to peripheral tissues. Following the release of free fatty acids (FFA) from VLDL by lipoprotein lipase (LPL), VLDL are converted into VLDL remnants, intermediate density lipoproteins and finally into low density lipoproteins (LDL).

Another important source of fat is our food. Dietary fat enters the circulation in chylomicrons, which are triglyceride-rich lipoproteins, synthesized by the small intestine. On the vascular endothelium, LPL releases FFA from chylomicrons. Subsequently, FFA can be taken up by tissues, such as skeletal muscle and adipose tissue. Once FFA enter peripheral tissues, they are converted to acetyl CoA and directed into one of the following metabolic pathways; 1) incorporation into lipids (e.g. triglycerides or phospholipids) or 2) mitochondrial β-oxidation. Oxidation of FFA provides energy in an efficient manner and is quantitatively important, especially under fasting conditions.

During starvation, as well as during exercise or periods of stress, FFA will be released from adipocytes via lipolysis. Lipolysis can be defined as the hydrolysis of triacylglycerol to glycerol and FFA molecules. In adipose tissue, lipolysis is controlled by hormone-sensitive
lipase (HSL) and adipose triglyceride lipase (ATGL). Once liberated by lipolysis, FFA have 2 possible fates: they can be transported in plasma bound to albumin and subsequently delivered to different organs for oxidation or re-esterification to form triacylglycerol.\textsuperscript{12,13}

1.2.3 Methods to measure glucose production, gluconeogenesis and lipolysis

**Endogenous glucose production:**

The rate of appearance ($R_a$) of glucose represents the sum of endogenous glucose production and exogenous glucose supply. The $R_a$ of glucose can be measured in human subjects in vivo with the use of stable glucose isotopes. In this thesis, we use [6,6-$^2$H$_2$]-glucose, i.e. ‘labelled’ glucose (the tracer), carrying an extra neutron on each of the 2 hydrogens on position C6 of glucose. The basic principle involved is that the $R_a$ of unlabelled glucose (the tracee) is determined by the dilution of infused labelled glucose. This procedure involves a priming dose, followed by a continuous infusion of labelled glucose. The purpose of the priming dose is to label the whole glucose pool and to reach the desired ratio of labelled versus unlabelled glucose within reasonable time. Subsequently, the priming dose can be determined by multiplying the desired enrichment (e.g. 1 %) with the glucose pool. The continuous infusion is necessary to maintain a constant ratio of labelled versus unlabelled glucose during the study.

When the steady state is reached, i.e. when the tracer/tracee ratio does not change during a certain time period, the $R_a$ of glucose can be calculated using the steady state equation:

$$R_a = \frac{TTR_{\text{tracer}} I_{\text{tracer}}}{TTR_p}$$

where $R_a$ = the rate of appearance of glucose in $\mu$mol/kg•min, $TTR_{\text{tracer}}$ = the tracer/tracee ratio of the tracer (± 100%), $I_{\text{tracer}}$ = the infusion rate of the tracer in $\mu$mol/kg•min and $TTR_p$ = the tracer/tracee ratio of plasma.

When steady state is not achieved, e.g. during a hyperinsulinemic-euglycemic clamp, the equation has to be modified:

$$R_a(t) = \frac{TTR_{\text{tracer}} I_{\text{tracer}}}{TTR_p(t)} + \frac{TTR_{\text{exo}} I_{\text{exo}}}{TTR_p(t)} - \frac{pVC(t) dTTR_p(t)}{dTTR_p(t)}$$
where \( t \) = the time of blood sampling, \( TTR_p(t) \) = the tracer/tracee ratio of plasma taken as the average of 2 consecutive samples, \( TTR_{exo} \) = tracer/tracee ratio of exogenous glucose, \( I_{exo} \) = the infusion rate of exogenous glucose in \( \mu\text{mol/kg} \cdot \text{min} \), \( p \) = the pool fraction, \( V \) = the distribution volume of glucose in \( \text{mL/kg} \) (± 40 mL/kg), \( C(t) \) = the plasma glucose concentration taken as the average of 2 consecutive samples in \( \text{mmol/L} \), \( dTTR_p(t)/dt \) = the change in the tracer/tracee ratio in plasma between 2 consecutive samples. All percentages are corrected for the background enrichment by subtraction of the basal enrichment.

Endogenous glucose production in the (non) steady state can be determined using the \( R_{\text{d}} \):

\[
\text{EGP}(t) = Ra(t) - I_{\text{tracee}}
\]

where \( \text{EGP}(t) \) = the endogenous glucose production rate in \( \mu\text{mol/kg} \cdot \text{min} \) and \( I_{\text{tracee}} \) = the infusion rate of unlabelled glucose in \( \mu\text{mol/kg} \cdot \text{min} \).

The rate of disposal of glucose \( (R_d) \) can be calculated using the non-steady state equation:

\[
R_d(t) = R_{a}(t) - pV \frac{dC(t)}{dt}
\]

where \( R_d \) = the rate of glucose disposal in \( \mu\text{mol/kg} \cdot \text{min} \) and \( dC(t)/dt \) = the change in the unlabelled plasma glucose concentration between 2 consecutive samples in \( \text{mmol/L} \). \(^{14, 15}\)

**Gluconeogenesis**

Gluconeogenesis can be measured using a variety of techniques. In this thesis, we applied the deuterated water \( (^2\text{H}_2\text{O}) \) method. This method is based on the ingestion of the stable isotope \( ^2\text{H}_2\text{O} \) with subsequent measurement of plasma water \( ^2\text{H}_2\text{O} \) enrichment. During gluconeogenesis, deuterium attaches to carbon 5 of glucose (C5). Glucose molecules produced by gluconeogenesis and glycogenolysis are labelled with deuterium at carbon 2 of glucose (C2). Hydrogen from plasma water is added to C2 of glucose-6-phosphate in the conversion from fructose-6-phosphate during gluconeogenesis. Glucose-6-phosphate is also formed as an intermediate during glycogenolysis and equilibrates extensively with fructose-6-phosphate, resulting in the exchange of hydrogen at C2 of glucose-6-phosphate with that in body water. The ratio of C5 and C2 enrichment of glucose equals fractional gluconeogenesis \(^{16-18}\). It was shown that after equilibration the enrichment at the C2 position of glucose equals the enrichment of total body water. Because the analysis of total
body water enrichment is considerably less complicated than analysis of $C_2$ enrichment of glucose, measurement of enrichment in total body water is preferred 18.

**Lipolysis:**
Lipolysis can also be measured in a non-invasive way in human subjects in vivo with the use of stable isotopes. In this thesis, $[^2H_5]$-glycerol was applied. The $R_a$ of unlabelled glycerol is determined by the dilution of infused $[^2H_5]$-glycerol and reflects lipolysis. The methods to determine the $R_a$ of glycerol in the (non) steady state are comparable to those of endogenous glucose production 15.

1.2.4 Regulation of glucose and lipid metabolism
The ‘classical’ hormones involved in the regulation of glucose and lipid metabolism are:

**Insulin:**
Insulin is considered to be the most important hormone controlling glucose metabolism. Insulin is produced by the $\beta$-cells of the pancreas. In healthy subjects, insulin suppresses endogenous glucose production by inhibiting both glycogenolysis and gluconeogenesis. In addition, insulin suppresses lipolysis and protein breakdown, while it stimulates de novo lipogenesis, protein synthesis and peripheral glucose disposal, the latter predominantly into skeletal muscle via the glucose transporter-4 (GLUT-4). The influence of insulin on these different metabolic processes depends to a considerable degree on the plasma insulin concentration. Lipolysis has the highest insulin sensitivity with a maximum suppression at an insulin concentration of $\pm$ 200 pmol/L 19, followed by endogenous glucose production, which is completely inhibited at an insulin concentration of $\pm$ 400 pmol/L 20. Peripheral glucose disposal as well as protein synthesis are maximally stimulated at an insulin level of $\pm$ 500 pmol/L, which is $\pm$ 7 times the physiological upper limit of the fasting plasma insulin concentration 20, 21.

**Glucagon:**
Glucagon is produced by the pancreatic $\alpha$-cells and its effects on glucose metabolism are opposite to the effects of insulin. Glucagon increases both hepatic glycogenolysis as well as gluconeogenesis. In healthy humans, glucagon has no major effect on peripheral glucose disposal 12.
Catecholamines:
The catecholamines epinephrine and norepinephrine normally do not play an essential role in the regulation of glucose and lipid metabolism under basal conditions. However, in situations of stress and during exercise the catecholamines become important in preventing hypoglycemia. Epinephrine and norepinephrine both increase glucose levels via stimulation of glycogenolysis as well as gluconeogenesis and via a sustained suppression of glucose disposal. The catecholamines also stimulate lipolysis, which results in an increase in the release of FFA. These FFA could function as alternative fuel sources for glucose\textsuperscript{10, 12}.

Cortisol:
Cortisol has a stimulatory effect on hepatic glucose production via an increase in both glycogenolysis and gluconeogenesis. In contrast to glucagon and the catecholamines, this effect is much slower and takes several hours to occur. Cortisol also induces insulin resistance both at the level of the liver as well as peripherally, resulting in increased hepatic glucose production and in decreased peripheral glucose disposal. Regarding lipid metabolism, cortisol has a stimulating effect on lipolysis\textsuperscript{22, 23}.

Growth hormone:
Growth hormone predominantly affects metabolism during prolonged starvation. This hormone induces hepatic and peripheral insulin resistance. Like cortisol, these effects on glucose metabolism take place after several hours. Growth hormone also stimulates lipolysis to provide substrates for gluconeogenesis and alternative fuel sources for glucose during prolonged fasting\textsuperscript{24}.

FFA:
Besides being energy substrates, FFA are metabolic messengers as well. FFA play a central role in glucose metabolism in both healthy individuals and in insulin resistant patients. In healthy subjects, FFA have a stimulatory effect on gluconeogenesis but not on hepatic glucose production, due to a concomitant decrease in glycogenolysis\textsuperscript{25}. In addition to these direct effects on gluconeogenesis, FFA also inhibit insulin-mediated suppression of endogenous glucose production by inhibiting insulin-mediated suppression of glycogenolysis\textsuperscript{26}. Regarding peripheral glucose disposal, FFA induce insulin resistance as well. It is thought that FFA (metabolites) inhibit insulin-stimulated glucose transport via alterations in the insulin signaling pathway\textsuperscript{27}. During starvation, FFA-induced insulin
resistance is physiological: it preserves carbohydrates for use by vital organs, such as the central nervous system.

Besides these ‘classical’ hormones, several other factors influence glucose and lipid metabolism as well. Important regulators of metabolism are the (adipo)cytokines. The role of the adipocytokines in lipid and glucose metabolism will be discussed below. The cytokines play a major role in metabolism during infections and in diseases in which inflammation is induced:

**TNF-α:**
In human subjects as well as in rodents, TNF-α induces insulin resistance via an increase in lipolysis and via inhibition of the insulin signaling pathway, leading to a decrease in the translocation of GLUT-4 \(^{28-30}\). However, as TNF-α-neutralizing antibodies failed to improve insulin sensitivity in type 2 diabetes, the role of TNF-α remains to be defined in human subjects \(^{31}\).

**IL-1:**
In human adipocytes as well as in rats IL-1 induces hepatic and peripheral insulin resistance \(^{32,33}\). There are no data on the effects of IL-1 on glucose metabolism in human subjects.

**IL-6:**
In rodents, central administration of IL-6 increases energy expenditure, whereas peripheral administration induces hepatic and peripheral insulin resistance as well as dyslipidemia \(^2,34\). In human volunteers, the data about its effect on glucose metabolism are conflicting \(^{30,35-38}\).

1.3 Adipose tissue

Adipose tissue can be divided into 2 major compartments, subcutaneous (SAT) and visceral adipose tissue (VAT), which vary both in their distribution and metabolism. The subcutaneous and visceral compartments constitute ± 80% and 10% of total body fat respectively, with other depots such as retroperitoneal and perirenal fat accounting for the remainder \(^{28}\).
Both SAT as well as VAT consists of 2 types of adipose tissue, namely brown and white adipose tissue. The predominant type of adipose tissue is white. Brown adipose tissue is mainly involved in heat production through thermogenesis, a process mediated by mitochondrial uncoupling protein 1. This is a crucial process in several animals as well as in human newborns, whose brown fat depots are therefore abundant. However in humans, shortly after birth, brown fat is largely replaced by white adipose tissue as its role in thermogenesis becomes less important. White adipose tissue has several important functions: besides its role in mechanical cushioning, it consists of cells which are specialized in triglyceride synthesis and storage. By compact storage of triglycerides, white adipose tissue can provide energy during sustained periods of fasting via an increase in lipolysis. On the other hand, during the postprandial period, white adipose tissue is able to buffer the lipids that are released from triglycerides-rich lipoproteins. In addition to these functions, white adipose tissue has recently been recognized as a highly active metabolic and endocrine organ which is capable of producing a variety of hormones, including TNF-$\alpha$, IL-6, resistin, leptin and adiponectin. Some of these adipocytokines mainly originate from adipocytes (leptin, adiponectin) while others are shared with other cells, such as macrophages and endothelial cells (TNF-$\alpha$, IL-6). The production and secretion of the various adipocytokines differ between VAT and SAT. Visceral fat has been described to secrete more IL-6 and adiponectin than subcutaneous fat. In contrast, leptin expression and secretion is higher in SAT than in VAT. The adipocytokines have a major influence on glucose and lipid metabolism. Adiponectin appears to be the most interesting adipocytokine by its insulin-sensitizing effects and its potent role in the pathogenesis of disturbances in glucose metabolism.

1.4 Adiponectin

Adiponectin is an abundant circulating plasma protein, predominantly produced by adipocytes. In healthy rodents, administration of adiponectin ameliorates glucose metabolism by enhancing peripheral glucose uptake and suppressing hepatic glucose production. Moreover, in both lipoatrophic and obese animals, adiponectin reverses insulin resistance. These effects occur via activation of AMPK in muscle, adipocytes as well as in the liver. This results in stimulation of FFA oxidation and consequently a reduction in triglycerides. In plasma, adiponectin circulates as several different entities, including a HMW (high-molecular-weight), a hexameric (medium-molecular-weight) and a trimeric (low-molecular-weight) form. The HMW oligomer has been implicated as the most active
form, responsible for the insulin-sensitizing effects of adiponectin in the liver and skeletal muscle 49.

Plasma levels of adiponectin, primarily of the HMW form, are reduced in insulin resistant subjects with obesity, type 2 diabetes mellitus and HIV-associated lipodystrophy 6, 7, 50, 51. Considering the insulino-mimetic properties of adiponectin, the reduction in adiponectin levels could play a role in the pathogenesis and/or perseverance of the disturbances in glucose and lipid metabolism in such patients.

Sepsis or other acute infections, such as malaria, are frequently complicated by disturbances in glucose metabolism as well. Plasma adiponectin levels and the involvement of adiponectin in the derangements in glucose homeostasis during acute infections have not been investigated yet.

1.4.1 Regulation of adiponectin

Since hyperinsulinemia and hyperglycemia are characteristic biochemical features of insulin resistant patients, insulin as well as glucose could be involved in the down-regulation of adiponectin. The effects of insulin have been investigated extensively. Most, but not all studies 52 have reported an increase in adiponectin expression and secretion in 3T3-L1 adipocytes in response to insulin 53-56. Moreover, insulin stimulates adiponectin secretion via the phosphatidylinositol 3-kinase (PI3K)-dependent signaling pathway, since selective inhibition of this pathway prevented the effect of insulin 55, 57. Besides the PI3K-pathway, insulin mediates its metabolic effects via 2 other main signal transduction pathways: the Mitogen-activated protein kinase (MAPK) pathway and in synergy with amino acids the mammalian target of rapamycin (mTOR) pathway. The influence of the MAPK- and mTOR pathway on adiponectin production and secretion have not yet been studied.

In contrast to these studies in 3T3-L1 adipocytes, insulin decreases plasma adiponectin levels in human subjects 58-60. The cause for the different effects of insulin on adiponectin in 3T3-L1 adipocytes compared to human subjects remains to be elucidated.

The effects of glucose on adiponectin levels have been investigated only in rats. Hyperglycemia resulted in enhanced adiponectin expression in visceral fat, whereas plasma levels remained constant 61. The regulation of plasma adiponectin levels by glucose has not been assessed in human subjects. In addition, studies on the influence of the combination of hyperinsulinemia and hyperglycemia on human plasma adiponectin levels are lacking.

1.4.2 Disturbances in glucose and lipid metabolism and the role of
**adiponectin**
The most common perturbation of glucose metabolism is insulin resistance. Insulin resistance is defined as a reduced action of insulin in target tissues, i.e. skeletal muscle, adipocytes and the liver. The most important metabolic consequences of insulin resistance are an increase in hepatic glucose production as well as in lipolysis and a reduction in peripheral glucose disposal. In the early stages of insulin resistance, plasma glucose is normal and insulin levels are moderately increased. Progression of insulin resistance in combination with pancreatic β-cell failure lead to overt type 2 diabetes mellitus 12.

**Obesity and type 2 diabetes mellitus:**
There is a strong association between insulin resistance and obesity, especially with abdominal obesity. An estimated 60-90% of patients with type 2 diabetes mellitus are or have been overweight. In addition, in the early stages of obesity, insulin resistance is already apparent 62. The pathophysiological mechanisms underlying the relation between obesity and insulin resistance have not been fully elucidated. Ectopic fat storage has been suggested to be one of the key mechanisms. An excess of lipids, exceeding the capacity of subcutaneous adipocytes, results in a lipid flux to visceral fat and eventually to surrogate storage depots such as in the liver and skeletal muscle.

Visceral fat is more strongly associated with perturbations in glucose and lipid metabolism than subcutaneous fat. Compared to subcutaneous adipocytes, lipolytic activity is higher in visceral adipocytes, due to a more pronounced effect of β-adrenergic stimulation and less sensitivity to the antilipolytic effects of insulin. Moreover, an increase in visceral fat leads to a greater FFA delivery directly to the liver due to the drainage of visceral fat into the portal vein 28, 41. A high influx of FFA (metabolites) into the liver negatively influences glucose and lipid metabolism with consequently hepatic steatosis and hepatic insulin resistance 63, 64. Intramyocellular lipid accumulation negatively affects glucose metabolism as well. Via inhibition of the insulin signaling cascade, FFA and its metabolites (e.g. diacylglycerol and ceramides) reduce insulin-mediated glucose disposal into muscle 63-65.

Besides induction of ectopic lipid deposition, obesity also impairs the endocrine function of adipocytes, resulting in disturbances in the synthesis and secretion of adipocytokines 28, 41. Furthermore, hypertrophied adipocytes induce a local inflammatory reaction with infiltration of macrophages and overexpression of cytokines and chemokines 42, 66. Both factors probably contribute to an increase in plasma (adipo)cytokines with a deleterious
effect on metabolism, including TNF-α and IL-6 in obese humans. These (adipo) cytokines interfere with normal insulin signaling and eventually lead to insulin resistance.

In contrast to the increased levels of these proteins, plasma adiponectin levels are decreased in obese subjects. In addition, patients with type 2 diabetes mellitus have reduced adiponectin levels as well, primarily of the most active HMW form. Considering the insulin-sensitizing effects of adiponectin, the decrease in adiponectin levels in patients with obesity and type 2 diabetes could play a role in the etiology of the disturbances in glucose and lipid metabolism in these patients. Indeed, low total and HMW plasma concentrations of adiponectin are associated with insulin resistance.

Lipodystrophy:
The most common forms of lipodystrophy are genetic and HIV-associated lipodystrophy. Lipodystrophy is characterized by a total or partial loss of peripheral subcutaneous fat (lipoatrophy) often accompanied by accumulation of visceral, breast and dorsocervical (buffalo hump) fat. In addition to visceral fat accumulation, lipoatrophy is associated with insulin resistance as well. Due to the loss of SAT, the capacity to store lipids is limited. This leads to ectopic fat deposition. In addition, the endocrine function of adipocytes is impaired as well in lipoatrophy, resulting in disturbances of adipocytokine levels. Similar to the situation in obese humans, these factors could be involved in the etiology of the disturbances in glucose and lipid homeostasis in lipodystrophic patients.

Certain antiretroviral drugs used for the treatment of HIV-infection have been demonstrated to play a major role in the pathogenesis of HIV-lipodystrophy. The currently most commonly used antiretrovirals belong to 1 of 3 classes, i.e. protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI). Besides inducing metabolic disturbances via changes in body fat distribution, antiretrovirals have also been reported to influence metabolism in a direct way.

Regarding the role of PI, prospective studies showed a decrease in insulin sensitivity and β-cell dysfunction in HIV-1-infected patients several months after starting a PI-containing regimen. Moreover, in healthy volunteers, administration of a single dose of the PI indinavir reduced peripheral glucose uptake. In-vitro research has demonstrated that
several PI are able to acutely inhibit the activity of GLUT-4, thereby offering a possible explanation for the PI-induced insulin resistance in human subjects. Furthermore, PI have been shown to alter the secretion of adipocytokines and to enhance basal lipolysis, which could both cause disturbances in metabolism.

NRTI are thought to contribute mainly indirectly to disturbances in metabolism by inducing changes in body fat distribution and lipoatrophy in particular. However, as insulin resistance has been described to develop as early as 4 weeks after starting a regimen of 2 NRTI, NRTI may also disturb glucose metabolism more directly. Finally, NRTI have also been described to disturb the production and secretion of adipocytokines.

In contrast to PI and NRTI, NNRTI have not been demonstrated to negatively influence glucose or lipid metabolism.

Prospective studies, investigating the contribution of individual drug classes, NRTI in particular, and the sequence of onset of the derangements in glucose and lipid metabolism as well as in body fat distribution are lacking in antiretroviral drug-naive, HIV-1-infected patients starting antiretroviral therapy.

Similar to what is the case in obese and diabetic patients, adiponectin could contribute to the perturbations in glucose and lipid metabolism in HIV-lipodystrophic patients. Plasma adiponectin levels are decreased in HIV-lipodystrophic patients compared to HIV-positive patients without lipoatrophy and compared to healthy subjects. Moreover, the total plasma adiponectin levels are positively correlated to insulin sensitivity. Whether a decrease in plasma adiponectin levels precedes the onset of disturbances in glucose and lipid metabolism in HIV-lipodystrophic patients is unknown.

The effects of PPAR-γ agonists in HIV-lipodystrophic patients have been explored by several studies. However, the majority of these studies focused on the influence of PPAR-γ agonists on body fat distribution and therefore did not examine glucose homeostasis in detail. The association between a PPAR-γ agonist-induced increase in adiponectin levels and changes in insulin sensitivity at the level of peripheral glucose disposal, hepatic glucose production and lipolysis remains to be elucidated in HIV-lipodystrophy.

Infections:
Acute infections, such as sepsis and malaria, are frequently complicated by disturbances in glucose metabolism. Hypoglycemia is seen in critical illness, as an infrequent feature of early sepsis. In animal studies, it was demonstrated that hypoglycemia during early sepsis is the consequence of a decrease in glucose production by the liver combined with a relative stimulation of glucose disposal by selective macrophage-rich tissues, mostly...
by an insulin-independent mechanism. The pathophysiology of hypoglycemia can not be explored in humans as immediate treatment is mandatory. Sepsis can be mimicked in healthy humans via intravenous administration of Gram-negative bacterial lipopolysaccharide (LPS). Studies with LPS in humans are extremely scarce and confirm early hypoglycemia, but did not explore its mechanism. Data on peripheral and hepatic insulin sensitivity in early sepsis or after LPS administration in human subjects are lacking.

A prolonged duration of sepsis is associated with an acute and reversible state of insulin resistance, leading to hyperglycemia. Hyperglycemia could be the consequence of a decline in peripheral glucose uptake in combination with an increase in endogenous glucose production as well as lipolysis during sepsis. Counterregulatory hormones and pro-inflammatory cytokines have been hypothesized to contribute to these disturbances. The role of adiponectin in the metabolic derangements during sepsis is unknown.

In patients with severe malaria, hypoglycemia is a frequent complication, occurring in 8–30% of individuals affected with this disease, particularly in those with cerebral malaria. As adiponectin suppresses hepatic glucose production, adiponectin could be involved in the etiology of hypoglycemia during malaria. The potential pathophysiological role of adiponectin in the development of hypoglycemia in malaria patients has not been assessed yet.

1.5 Outline of this thesis

This thesis examines some aspects regarding the regulation of adiponectin and its role in the disturbances in glucose and lipid metabolism with an emphasis on patients with infections and HIV-lipodystrophy.

Chapter 2

Insulin resistant patients are characterized by high plasma insulin levels. Insulin could be responsible for the down-regulation of adiponectin in these patients. Insulin mediates its metabolic effects via 3 different signal transduction pathways: the PI3K- pathway, the MAPK pathway and in synergy with amino acids the mTOR pathway. We examined the influence of these different insulin signaling cascades on the production and secretion of adiponectin in 3T3-L1 adipocytes. In addition, we also investigated whether autophagosomal breakdown regulates adiponectin levels.
Chapter 3
Besides hyperinsulinemia, patients with type 2 diabetes mellitus also have high plasma glucose concentrations. Hyperinsulinemia, hyperglycemia or its combination could be involved in the etiology of low adiponectin levels. In chapter 3 we investigated the selective effects of insulin, glucose and its combination on plasma (HMW) adiponectin levels in healthy subjects.

Chapter 4
Sepsis and other acute infections are often associated with disturbances in glucose homeostasis. As no data on peripheral and hepatic insulin sensitivity in early sepsis in humans exist, we studied glucose metabolism during hyperinsulinemic-euglycemic clamps with the use of stable isotopes in healthy volunteers after LPS administration and in a control setting. The effects on plasma adiponectin levels were examined as well.

Chapter 5
Disturbances in glucose metabolism frequently complicate severe malaria. We investigated plasma adiponectin levels in malaria patients and correlated these levels to endogenous glucose production rates to assess the role of adiponectin in the regulation of glucose production in malaria.

Chapter 6 and 7
Patients with antiretroviral therapy-associated lipodystrophy are characterized by changes in body fat distribution as well as by derangements in lipid and glucose metabolism. In order to obtain more insight into the contribution of individual drug classes, NRTI in particular, and into the sequence of onset of metabolic disturbances, we studied body composition and metabolism in detail in antiretroviral therapy-naive, HIV-1-infected patients starting treatment with a NRTI-containing regimen (lopinavir/ritonavir + zidovudine/lamivudine (LPV/r + AZT/3TC)) or a NRTI-sparing regimen (lopinavir/ritonavir + nevirapine (LPV/r + NVP)). The patients were studied at baseline and 3 (chapter 6), 12 and 24 (chapter 7) months following the start of treatment.

Chapter 8
Considering the insulino-mimetic properties of adiponectin, up-regulation of plasma adiponectin could result in improved glucose and lipid metabolism in HIV-associated lipodystrophy. In chapter 8, we examined the effects of a rosiglitazone-induced increase in adiponectin levels on insulin sensitivity by performing hyperinsulinemic-euglycemic
clamps using stable isotopes at baseline and 16 weeks after starting treatment with rosiglitazone in a double-blind placebo-controlled trial.

Chapter 9

From the start until the end of this thesis the amount of publications in the Medline database, which mention adiponectin have increased exponentially from ± 100 to 4000. In chapter 9 we summarize and put into perspective recent advances regarding the regulation of adiponectin and its role in lipid and glucose metabolism.

1.6 Reference list

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