A characterisation of low-grade inflammation and metabolic complications in HIV-infected patients

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This thesis is based on the following publications

I. Lipodystrophy in human immunodeficiency virus patients impairs insulin action and induces defects in beta-cell function.
Andersen, O., Haugaard, S. B., Andersen, U. B., Friis-Møller, N., Storgaard, H., Vølund, A., Nielsen, J. O., Iversen, J. & Madsbad, S. 2003: Metabolism. 52, 10, s. 1343-1353.

II. Enhanced glucagon-like peptide-1 (GLP-1) response to oral glucose in glucose-intolerant HIV-infected patients on antiretroviral therapy
Andersen, O*., Haugaard, S. B*., Holst, J. J., Deacon, C. F., Iversen, J., Andersen, U. B., Nielsen, J. O. & Madsbad, S. 2005: HIV Medicine. 6, 2, s. 91-8.

III. Circulating sex hormones and gene expression of subcutaneous adipose tissue oestrogen and alpha-adrenergic receptors in HIV-lipodystrophy: implications for fat distribution
Andersen, O., Pedersen, S. B., Svenstrup, B., Hansen, B. R., Paulsen, S. K., Rathje, G. S., Richelsen, B., Nielsen, J. O., Madsbad, S., Iversen, J. & Haugaard, S. B. 2007: Clinical Endocrinology. 67, 2, s. 250-8.

IV. Tumor necrosis factor alpha is associated with insulin-mediated suppression of free fatty acids and net lipid oxidation in HIV-infected patients with lipodystrophy
Haugaard, S. B*., Andersen, O*., Pedersen, E. S., Dela, F., Fenger, M., Richelsen, B., Madsbad, S. & Iversen, J. 2006: Metabolism. 55, 2, s. 175-82.

V. suPAR associates to glucose metabolic aberration during glucose stimulation in HIV-infected patients on HAART
Andersen, O., Eugen-Olsen, J., Kofoed, K., Iversen, J. & Haugaard, S. B. 2008: Journal of Infection. 57, 1, s. 55-63 8.

VI. Soluble urokinase plasminogen activator receptor is a marker of dysmetabolism in HIV-infected patients receiving highly active antiretroviral therapy
Andersen, O., Eugen-Olsen, J., Kofoed, K., Iversen, J. & Haugaard, S. B. 2008: Journal of Medical Virology. 80, 2, s. 209-16.

VII. Circulating soluble urokinase plasminogen activator receptor predicts cancer, cardiovascular disease, diabetes and mortality in the general population
Eugen-Olsen, J. *, Andersen, O.*, Linneberg, A., Ladelund, S., Hansen, T. W., Langkilde, A., Petersen, J., Pielak, T., Møller, L. N., Jeppesen, J., Lyngbæk, S., Fenger, M., Olsen, M. H., Hildebrandt, P. R., Borch-Johnsen, K., Jørgensen, T. & Haugaard, S. B. 1 sep 2010 : Journal of Internal Medicine. 268, 3, s. 296-308.

VIII. Different growth hormone sensitivity of target tissues and growth hormone response to glucose in HIV-infected patients with and without lipodystrophy
Andersen, O*., Haugaard, S. B*., Hansen, B. R., Orskov, H., Andersen, U. B., Madsbad, S., Iversen, J. & Flyvbjerg, A. 2004: Scandinavian Journal of Infectious Diseases. 36, 11-12, s. 832-9.

IX. Low-dose growth hormone and human immunodeficiency virus-associated lipodystrophy syndrome: a pilot study
Andersen, O., Haugaard, S. B., Flyvbjerg, A., Andersen, U. B., Ørskov, H., Madsbad, S., Nielsen, J. O. & Iversen, J. 2004: European Journal of Clinical Investigation. 34, 8, s. 561-568.

X. Sustained low-dose growth hormone therapy optimizes bioactive insulin-like growth factor-I level and may enhance CD4 T-cell number in HIV infection
Andersen, O., Hansen, B. R., Troensegaard, W., Flyvbjerg, A., Madsbad, S., Ørskov, H., Nielsen, J. O., Iversen, J. & Haugaard, S. B. 2010: Journal of Medical Virology. 82, 2, s. 197-205 8.

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the Department of Infectious Diseases, Hvidovre Hospital and the endurance and high compliance of all of the patients participating with great enthusiasm to help future patients from getting HIV-associated lipodystrophy.

CONTENTS

ABBREVIATIONS

AT	adipose tissue
cART	combined antiretroviral therapy
CVD	cardiovascular disease
DEXA	dual energy X-ray absorptiometry
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulphate
FFA	free fatty acids
GH	growth hormone
GIP	glucose-dependent insulin releasing polypeptide
GLP-1	glucagon-like peptide-1
GT	glucose tolerance
HAART	highly active antiretroviral therapy
HALS	HIV-associated lipodystrophy syndrome
HDL	high density lipoprotein
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IR	insulin resistance
LDL	low density lipoprotein
NOGM	non-oxidative glucose metabolism
NRTI	nucleoside reverse transcriptase inhibitor
OGTT	oral glucose tolerance test
PAI-1	plasminogen activator inhibitor type-1
PI	protease inhibitor
PI3-K	phosphatidyl inositol 3-kinase
PPAR	peroxisome proliferator-activated receptor
rhGH	recombinant human growth hormone
sTNFR	soluble tumour necrosis factor receptor
suPAR	Soluble urokinase plasminogen activator receptor
TNF	tumour necrosis factor

1. HIV AND HIV-ASSOCIATED LIPODYSTROPHY SYNDROME

Today, the life expectancy of asymptomatic HIV-infected patients who are still treatment-naive and have not experienced any HIV AIDS defining symptoms approaches that of non-infected individuals (van Sighem et al., 2010). A decade ago a 25-year-old Danish HIV-positive patient could expect to live 39 years compared to 51 years in the general population (Lohse et al., 2007). This steadily improvement comes from the introduction of novel treatment options in the mid-90s, the combined antiretroviral therapy (cART). Two studies in 1998 showed a dramatic decrease in HIV-related morbidity and mortality (Mocroft et al., 1998; Palella et al., 1998). However, not only improvements, but also new challenges, in the treatment of HIV became apparent. In 1997, an HIV-positive woman treated with indinavir presented with fat redistribution and hypertrophy of the breasts (Herry et al., 1997). In 1998, Carr and his colleagues described a new syndrome in HIV patients receiving highly active antiretroviral therapy (HAART) (Carr et al., 1998). The syndrome is called HIV-associated lipodystrophy syndrome (HALS) (Figure 1) and is characterised by fat redistribution and metabolic abnormalities, including dyslipidaemia and impaired glucose tolerance (GT). HIV-associated lipodystrophy syndrome has since caused great concern among both patients and doctors because of the highly visible and undesirable changes in body composition and the metabolic disturbances.

Figure 1: Photo of HIV-associated lipodystrophy syndrome with legend explaining atrophy and hypertrophy (with permission).

The prevalence of HALS varies greatly in the literature (7-83%), mainly due to a lack of homogenous criteria for defining the syndrome, as well as differences in age, sex, race, and treatment modalities in the described cohorts (Bernasconi et al., 2002; Justina et al., 2014; Loonam and Mullen, 2012; Saint-Marc et al., 2000).

In 2005 Bonnet proposed an objective index based on DEXA for the identification of lipodystrophy, the fat mass ratio. The fat mass ratio was defined as the ratio of percent trunk fat mass to percent lower limb fat mass. The proposed cutoff value was 1.5 for lipodystrophy, which corresponded to the mean plus one s.d. of fat mass ratio for HIV-negative men (Bonnet et al., 2005). Freitas and colleagues have later in a cross-sectional cohort study with 239 HIV-infected Caucasian men re-define lipodystrophy by fat mass ratio using higher cut-off values (Freitas et al., 2012). Besides fat mass ratio, waist thigh ratio, waist calf ratio, and arm to trunk ratio have been examined as objective measures for identification of HALS. The cutoffs values determined in different cross-sectional studies are highly dependent on the cohort examined (Beraldo et al., 2014). The scientific and clinical value of such measures and cut-off values still remain to be tested and validated in prospective settings before they can be applied and eventually implemented.

Up to date three major classifications have been proposed: Marrakech, MACS, and HOPS. The Marrakech classification is the only one that includes isolated metabolic abnormalities (Asensi et al., 2006). Any body fat modification is attributed to lipodystrophy. The HIV OutPatient Study (HOPS) allows a grading of the severity of lipodystrophy and yields a similar patient reported prevalence as Marrakech (Lichtenstein et al., 2001). The Multicenter AIDS Co-

hort Study (MACS) for the body composition substudy has the advantage that it excludes mild fat abnormalities, possibly connected with the physiological process of ageing (Brown et al., 2009). In a Danish study population, we have reported that 43% of HIV-positive patients on highly active antiretroviral therapy (HAART) have self-reported HALS (B. Hansen et al., 2009), which is consistent with the average found in other newly published trials where the prevalence of lipodystrophy was 32.4% (Justina et al., 2014).

In general, HALS is more frequent and more polymorphic in women than men, and the body fat changes observed in women do not only conform to the "android body habitus" characterised by increased truncal fat, but also a complex pattern of alterations with a striking degree of fat gain in the breasts, between the shoulders, and the side and front of the neck, and fat loss from the buttocks, face, arms, and lower limbs (Galli et al., 2003).

The index study by Carr in 1998 led to new studies that revealed potential host, disease, and treatment-related risk factors (Flint et al., 2009). Several pathogenic mechanisms for fat redistribution have been hypothesized and are summarised in Table 1. The proposed mechanisms are related primarily to specific HAART components and either syndrome-related lipoatrophy or lipohypertrophy or the metabolic disturbances.

For lipoatrophy, the mechanisms include the impairment of sterol regulatory element binding protein-1 (SREBP-1) regulating adipocyte differentiation(Carr, 2003a), inhibition of the respiratory function of the cell by nucleoside reverse transcriptase inhibition of polymerase-gamma, causing mitochondrial dysfunction (Brinkman et al., 1999, 1998) and adipocyte apoptosis. Adipocyte apoptosis may be mediated by pro-inflammatory cytokines, such as tumour necrosis factor (TNF)-α and IL-6 (Lihn et al., 2003), and the dysregulation of sex hormones (Paper III: Andersen et al., 2007).

For lipohypertrophy, the pathogenic mechanisms include a dysregulation of 11β-hydroxysteroid dehydrogenase (11β-HSD), causing local production of cortisol (Chen et al., 2002) and reducing plasma dehydroepiandrosterone sulphate (DHEAS) to cortisol (Piketty et al., 2001).

In addition, a dysregulation in the neuroendocrine axis towards a more sympathogenic pathway has been proposed, indicated by findings in a lipodystrophic patient showing higher levels of norepinephrine (Fliers et al., 2003).

HIV as a player in the development of HALS has gained more attention during the last few years, and novel evidence shows that 18 HIV-modulated proteins are significantly involved in disrupting some key processes. The interaction pathways of these HIV-modulated proteins enhance fatty acid synthesis, increase low density lipoprotein (LDL), dysregulate lipid transport, oxidize lipids, and alter cellular lipid metabolism without any influence from HAART (Rasheed et al., 2008).

1.1. GLUCOSE METABOLISM AND INSULIN RESISTANCE

Understanding the disturbances in glucose metabolism and implications in the HIV-positive individual requires a thorough understanding of glucose regulation in HIV-negative individuals, bearing

in mind that the consequence of insulin resistance (IR) in HIV-negative individuals do not result in the morphological changes observed in HIV-positive HAART-treated patients.

A key issue in the maintenance of normal life functions depends on a strict balance between glucose production, mainly from the liver (Kanemaki et al., 1998), glucose absorption in the intestine, and glucose uptake in peripheral tissues. The homeostasis is maintained primarily by insulin, which is secreted from pancreatic β-cells during food intake, and direct stimulation with incretins and signals from the central nervous system, such as acetylcholine (Hermans et al., 1987), arginine, and leucine (Trabelsi et al., 1995). The role of the gastrointestinal tract in influencing insulin secretion and glucose homeostasis has long been recognised. Zunz and La Barre first introduced the term "incretin" in reference to an insulin-stimulating hypoglycaemic factor found in duodenum extract (Zunz and Barre, 1929). Incretin hormones are gastrointestinal peptide hormones released in response to nutrient ingestion, intensifying the glucose-induced insulin response. Mainly, two peptide hormones, glucose-dependent insulin releasing polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), cause the incretin effect in man. K cells from the upper small intestine secrete GIP, whereas GLP-1 is mainly produced in the enteroendocrine L cells located in the distal intestine. The effects of these peptides are mediated through binding specific receptors, though part of their biological actions may also involve neural modulation. Glucagon-like peptide-1 exerts other significant actions in addition to the effects on insulin secretion, including the stimulation of insulin biosynthesis, inhibition of glucagon secretion, inhibition of gastric emptying and acid secretion, reduction of food intake, and trophic effects on the pancreas. Because the insulinotropic action of GLP-1 is preserved in type 2 diabetic patients, this peptide is a candidate therapeutic agent for diabetes (Gautier et al., 2005; Nauck, 2009).

Insulin stimulates cell growth and differentiation and is the key regulator of blood glucose, mainly by stimulating glucose uptake in skeletal muscle (glycogenolysis) and suppressing hepatic glucose production (gluconeogenesis). Insulin exerts its effect on glucose transport into cells after binding to its receptor via two independent pathways: the Mitogen-activated Protein (MAP) kinase and PI-3 kinase pathways. The PI-3 kinase is responsible for the major metabolic actions of insulin, the translocation and activity of the main glucose transporter, glucose transporter-4, in skeletal muscle and adipose tissue (AT) (Furtado et al., 2002) reviewed in (Kumar and O'Rahilly, 2005). Other factors that interfere with gluconeogenesis and glycogenolysis are: glucagon (Wahren and Ekberg, 2007), cathecholamines (Barth et al., 2007), corticosteroids (Bollen et al., 1998), GH (Segerlantz et al., 2003), and adiponectin (Capeau, 2007). An increase in IL-1β and IL-6, which represents a classic view of a low-grade inflammatory response, has been shown to inhibit insulin-stimulated glycogen synthesis in hepatocytes (Kanemaki et al., 1998) and to degrade glycerol (Petersen et al., 1996).

Tabel 1 Proposed pathogenic mechanisms for HALS

Factors involved	Mechanism	References and Comments
Protease Iinhibitors	1) A decrease in lipoprotein lipases activity (LPL) 2) Activation of Sterol-regulatory-element-binding-protein-1 and 2 3) Activation of peroxisome proliferator-activated receptor (PPAR)-γ 4) Inhibition of proteasomes 5) inhibition of GLUT-4	(Carr et al., 1998) (Flint et al., 2009)
Nucleoside reverse transcriptase inhibitors	1) Inhibit polymerase-γ and mitochondrial DNA	(Brinkman et al., 1998)
11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) mRNA	1) It has been hypothesised that local cortisol production in visceral adipose tissue of lipodystrophic HIV-infected patients might explain excess of this fat compartment.	(Chen et al., 2002) One study has found increased 11β-HSD1 in subcutaneous abdominal adipose tissue of lipodystrophic HIV-infected patients (Sutinen et al., 2004) However we did not find any difference in peripheral subcutaneous fat (Paper III: Andersen et al., 2007)
Ratio of circulating cortisol and dehydroepiandrosterone sulphate (DHEAS)	DHEAS is a ligand for and has been shown to increase expression of PPAR-α in subcutaneous adipocytes and to affect the β-oxidation pathway.	Reduces plasma DHEAS to cortisol ratio has been demonstrated in lipodystrophic HIV-infected patients (Piketty et al., 2001).
Sex hormones	Plasma levels of estradiol and testosterone are major regulators of fat distribution in man.	We report that relatively low limb fat mass and abdominal subcutaneous fat mass are associated with low levels of estradiol (Paper III: Andersen et al., 2007).
Neuro-endocrine axis	Adipose tissue in various compartments is both sympathetic and parasympathetic innervated which may impact upon the balance of catabolism and anabolism and thereby affect fat distribution.	There has been demonstrated increased plasma norepinephrine in HALS. The importance of this pathway for HIV-lipodystrophy has been reviewed in (Fliers et al., 2003)

Insulin resistance is usually defined as a reduced tissue reaction to circulating insulin and can develop to any of the actions attributed to insulin. No strict blood insulin concentration can be defined as a normal limit, but IR can be calculated by a homeostasis model assessment of IR (HOMA-IR) (Ferrannini et al., 1996; Stern et al., 2005) or by the gold standard method, hyperinsulinaemic euglycaemic clamp. Normoglycaemia is maintained by intravenously infusing insulin and suppresses hepatic insulin secretion (DeFronzo et al., 1979). Under these circumstances, skeletal muscle accounts for more than 70% of the glucose utilization(Hannele, 1993).

Insulin resistance in healthy elderly people exhibits increased liver and skeletal muscle fat and a 40% reduction in mitochondrial oxidative and phosphorylative activity compared with a matched, younger group (Petersen et al., 2003). However, even a 60% reduction in insulin receptor substrate-1 (IRS-1) does not result in a reduction in insulin signalling or activity, as the biological phenomenon is not linear but a sigmoid curve (Whitehead et al., 2001), and a 15-20% reduction in mitochondrial function does not reduce the oxidative process.

1.2. SEX HORMONES

Over the past several years, there have been discussions about how HIV disease develops in women and men, and research has shown sex differences in viral load (Anastos et al., 2000). During acute/early infection, women tend to have lower viral loads than men with the same or similar CD4+ cell counts (Fang et al., 1997). However, this difference appears to be overt only in the first 5 years of infection. No impact has been seen on disease progression overall (Chaisson et al., 1995).

Women, particularly overweight women, appear to be more likely to experience fatty liver (hepatic steatosis) and increases in lactic acid related to NRTI (Miller et al., 2000; Moyle et al., 2002).The risk for severe lactic acidosis appears to be greater among pregnant women who take both d4T and ddI (Carr, 2003b; Sarner and Fakoya, 2002). Inflammation of the pancreas (pancreatitis) may also be more common in women (Arenas-Pinto et al., 2003; Ofotokun and Pomeroy, 2003), however, other investigators have not been able to show any gender-related differences in the risk of developing hyperlactataemia (Marceau et al., 2003)

Examinations of the mechanisms underlying the results showed that endogenous sex hormones differentially modulate glycaemic status and the risk for type 2 diabetes in men and women. High testosterone levels are associated with a higher risk of type 2 diabetes in women but with a lower risk in men; the inverse association of SHBG with risk was stronger in women than in men (Ding EL et al., 2006).

Endogenous levels of oestradiol and progesterone fluctuate in the peripheral blood of premenopausal women during the reproductive cycle. Dr. Asin and colleagues recently presented a study in which they examined the effects of sex hormones on HIV-1 replication in peripheral blood mononuclear cells (PBMCs). They compared HIV-1 replication in PBMCs infected in the presence of mid-secretory (high) and mid-proliferative (low) concentrations or in the absence of oestradiol or progesterone. Mid-proliferative phase conditions were found, to increase, and mid-secretory phase conditions to decrease, HIV-1 replication. No significant effects on HIV-1 reverse transcription or on CCR5 expression were found. In addition, the hormonal regulation of the HIV-1 virale gene, Long Terminal Repeat (LTR) in the absence of the viral regulatory protein Tat was assessed. Mid-proliferative hormone levels (low) enhanced LTR activity, whereas mid-secretory (high) hormone concentrations reduced the activity of LTR. These findings demonstrate that, in HIV-1-infected cells, oestradiol and progesterone regulate HIV-1 replication, most likely by directly altering the transcriptional activation of HIV-1. An additional indirect mechanism of the regulation of cytokine and chemokine secretion by sex hormones cannot be excluded (Asin et al., 2008)

1.3. ADIPOSE TISSUE AND LOW-GRADE INFLAMMATION

The primary function of AT is to store energy as triglycerides during periods of excess energy and to release the energy as free fatty acids (FFA) and glycerol during fasting or starvation. However, AT consists of a variety of other active cells, vascular endothelial and smooth muscle cells, mast cells, and pre-adipocytes, that can convert to macrophages (Charrière et al., 2003) and recruit macrophages from the circulation (Koutnikova and Auwerx, 2001). These cell types produce a variety of peptides, adipokines (e.g., leptin, adiponectin, TNF-α, IL-6) that have endocrine, autocrine, and paracrine effects within the AT, in the skeletal muscles, liver, and brain. These adipokines play an important role in the regulation of energy homeostasis, metabolism (Brüünsgaard and Pedersen, 2003), and the immunological imbalance of inflammation. Within AT, TNF-α causes IR through the inactivation of serine phosphorylation of insulin receptor and its substrate insulin receptor substrate-1 (Hotamisligil et al., 1994), but also through an increase in circulating FFA, which is caused by the induction of lipolysis and stimulation of hepatic lipogenesis (Grunfeld and Feingold, 1991). In addition, AT has the ability to aromatize androgens to oestrogens (Simpson, 2000).

The disturbed balance between the pro-and anti-inflammatory mediators, favouring increased levels of pro-inflammatory adipokines, cytokines, and chemokines is the hallmark of the immunological state of low-grade inflammation (Kolb and Mandrup-Poulsen, 2005). The inflammatory cytokines TNF-α and IL-6 may inhibit pre-adipocyte differentiation by blocking the major transcription factor peroxisome proliferator-activated receptor (PPAR)-γ. This blocking contributes to lipolysis and fat atrophy in inflammatory diseases, cancer, and chronic infectious diseases (MacDougald and Mandrup, 2002). M1-macrophages have a pro-inflammatory cytokine profile and reactive oxygen species (ROS) scavenger ability. In addition to the classically activated M1-macrophages, alternatively activated M2-macrophages that possesses an anti-inflammatory cytokine profile, have been demonstrated in subcutaneous biopsies from lipodystrophic patients (Lumeng et al., 2007). The function of M2-macrophages is traditionally linked to increased scavenger receptor activity; pro-tumour functions, including the promotion of angiogenesis; matrix remodelling; and are a potential source of fibrosis-inducing cytokines. These cellular and molecular interactions are currently weakly defined however emerging data support the distinct phenotypic differences of macrophages demonstrate that macrophage polarization is regulated by specific epigenetic mechanisms. In addition novel roles for the histone methyltransferase as marker for classical activation has been described, providing new insights into macrophage polarization that could be helpful to distinguish macrophage activation states and the relevance of these seemingly contradicting pathways and how they act together (Kittan et al., 2013)

Over the past few decades, significant advances have been made in delineating key extracellular and intracellular stimulators of fat cell formation, or adipogenesis. The main focus in the literature has been on finding new specific inhibitors of adipogenesis (Harp, 2004). However, understanding the balance between the positive and negative regulators of adipogenesis and inflammation has important health-related implications for HIV-positive patients and patients with lipodystrophy. Insulin and glucocorticoids stimulate adipogenesis and GH. Glucocorticoids have both anti and proinflammatoric effects. The anti-inflammatory activity of glucocorticoids is attributed to the repression of pro-inflammatory genes through signal transduction by their steroid receptor. The mechanisms modulating the pro-inflammatory effects of glucocorticoids are still not understood (Cruz-Topete and Cidlowski, 2015).The lipolytic action also stimulates pre-adipocyte differentiation in vitro (Tominaga et al., 2002). In addition, the autonomous nervous system has a dual effect on adipocyte differentiation, with an increase in lipolysis through β-1 and β-2 receptors and a decrease via α-2 receptors (Lafontan and Berlan, 2003).

Adiponectin

The adipokine adiponectin is recognised as a key regulator of insulin sensitivity, principally the high molecular weight form, and inflammation in AT. Adiponectin is produced by white and brown AT and circulates in the blood at very high concentrations; it has direct actions in the liver and skeletal muscle with an ability to improve hepatic insulin sensitivity, increase fuel oxidation via an up-regulation of adenosine monophosphate-activated protein kinase activity, and decrease vascular inflammation. In contrast to other adipokines, adiponectin secretion and its circulating levels are inversely proportional to body fat content. Levels are further reduced in subjects with diabetes (Kubota et al., 2002) and coronary artery disease. Adiponectin antagonises many effects of TNF-α, which, in turn, suppresses adiponectin production. Smoking cessation is associated with increased plasma adiponectin levels in men with stable angina, suggesting that the significance of smoking cessation may be partly explained by the increase in adiponectin (Otsuka et al., 2009).

Leptin

The adipokine and hormone leptin has important effects on the regulation of body weight, metabolism, and reproductive function (Friedman and Halaas, 1998). Leptin is expressed predominantly by adipocytes, and a minor amount is secreted from endothelial cells. Leptin receptors are highly expressed in areas of the hypothalamus known to be important in regulating body weight, as well as T lymphocytes and vascular endothelial cells. The mechanisms responsible for regulating leptin expression in adipocytes are not known in detail but there is accumulating evidence that increased expressions and/or activities of the transcription factors C/EBPs and PPARs are necessary for the expression of adipocyte-specific genes and adipokines, including leptin and adiponectin (Park et al., 2014). A number of hormones are likely to modulate expression of the leptin gene, including glucocorticoids and insulin (Ahima and Osei, 2004). In addition to the effect on the hypothalamus, leptin acts directly on liver cells and skeletal muscle, stimulating the oxidation of fatty acids in the mitochondria (Park and Ahima, 2014). This action reduces the storage of fat in said tissues, but not in AT. Leptin also enhances the production of Th1 cells, promoting the inflammatory response (Procaccini et al., 2012).

The inflammatory syndrome

Adipose tissue, adipokines, and a vast majority of the proteins involved in coagulation and fibrinolysis might be looked upon as a common inflammatory syndrome. Together, these factors may contribute to the development of IR, type 2 diabetes and metabolic syndrome (Nix and Tien, 2014; Paula et al., 2013) and atherosclerosis related to the development of coronary artery disease (Jeppesen et al., 2007).

PAI-1

Plasminogen activator inhibitor type 1 (PAI-1) is an important inhibitor of fibrinolysis. Plasminogen activator inhibitor type-1 is increased in obese subjects and may play a particular role in this process as it has been shown to be an independent risk factor for CVD (Balagopal et al., 2011). The suppression of fibrinolysis due to high plasma concentrations of PAI-1 is associated with the development of myocardial infarction (Hamsten et al., 1987). In addition, high concentrations of tissue plasminogen activator and d-dimer, a measure of fibrinolysis, increase the risk of myocardial infarction (Ridker et al., 1994). The PAI-1 is a fast-acting inhibitor of plasminogen activation. The transcription and secretion of PAI-1 from endothelial cells is increased by Insulin, proinsulin-like molecules, glucose, very low density lipoprotein (VLDL), and triglycerides (Maiello et al., 1992). The association between the fibrinolytic system, AT, and sex hormones has been investigated in postmenopausal HIV-negative women receiving oestrogen replacement therapy. Treatment with oestrogen lowers plasma PAI-1 concentrations; premenopausal women have lower plasma PAI-1 levels than postmenopausal women (Gebara et al., 1995). The presumed cardioprotective effect of oestrogen in premenopausal women may be mediated, in part, through an increase in fibrinolysis, linking these areas together.

suPAR

Soluble urokinase Plasminogen Activator Receptor (suPAR) is a highly flexible pro-inflammatory molecule (Huai et al., 2006) with intrinsic chemotactic properties (Fazioli et al., 1997). Soluble urokinase plasminogen activator receptor is the soluble form of the urokinase plasminogen activator receptor (uPAR), which is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein expressed on the surface of various immunologically active cells, particularly monocytes/macrophages and T-lymphocytes, but also endothelial and smooth muscle cells and adipocytes (Smith and Marshall, 2010). The uPAR binds urokinase plasminogen activator (uPA) and localises the activation reactions in the proteolytic cascade of plasminogen activation (Behrendt, 2004). Plasminogen activator inhibitor type-1 has been shown to bind the receptor-bound uPA when uPAR is shedding its soluble form, suPAR, from the cell surface (Blasi and Carmeliet, 2002). The inactive PAI-1/uPA/uPAR complex is internalised by uPAR binding to Low Density Lipoprotein receptor-related protein (Czekay et al., 2001; Herz et al., 1992), suggesting a physiological role of uPAR as a lipoprotein receptor. In addition, uPAR has been shown to stimulate cell adhesion, cell migration, and intracellular signalling by interaction through its co-receptors integrin (Degryse et al., 2001) and vitronectin (Hillig et al., 2008; Madsen et al., 2007). Resnati et al demonstrated in 2001 (Resnati et al., 2002) that uPAR binds to a member of the seven transmembrane G-protein coupled receptor family formyl peptide receptor like-1, establishing the signalling leading to the uPAR-integrin association and thyrokinase activation. In addition, uPAR mediates cell migration through the activation of protein kinase C and phosphatidyl inositol 3-kinase (PI3-K)

(Ossowski and Aguirre-Ghiso, 2000). The expression of uPAR is elevated during inflammation and tissue remodelling, which frequently indicates poor prognosis (Smith and Marshall, 2010). The coordination of extracellular matrix proteolysis and cell signalling by uPAR underlies its important function in cell migration, proliferation, and survival and makes it an attractive therapeutic target in inflammatory diseases. Sidenius et al. were the first to demonstrate an important association between the plasminogen activator system and disease progression in HIV-1 infection (Sidenius et al., 2000)

Many investigational biomarkers suffer from fragility and, as such, are not a real option to use, were sampling schedule has to fit into a daily clinical routine setting. We tested the stability of suPAR in six HIV-infected patients during a 3-h oral glucose tolerance test (OGTT). Plasma suPAR exhibited a small CV (11%) during the OGTT (Paper V: Andersen et al., 2008b), and circadian suPAR measured every 20 min for 24 h in five patients did not show systematic fluctuations. The 24-h CV was similar to the inter-assay variance (Paper VI: Andersen et al., 2008a). A stable biomarker such as suPAR is a prerequisite for implementing the knowledge of biomarker research into daily practise.

A possible interaction between inflammation and GH has been proposed based on increased cardiovascular mortality in a scenensce-accelerated mice model (Forman et al., 2009) and observations of the acute phase protein, C-reactive protein (CRP). CRP is reduced in acromegalic patients before treatment and increased after treatment is initiated, suggesting that excess GH/insulin-like growth factor (IGF)-I has anti-inflammatory effects. In patients with GH deficiency, increased levels of the inflammatory biomarkers YKL-40, IL-6, and CRP have been reported (Andreassen et al., 2007). Together, these results indicate that the GH/IGF-I system is a suppressive regulator of inflammatory processes (Higashi et al., 2012).

1.4. GROWTH HORMONE AND RELATED PROTEINS

Since the beginning of the HIV era, treatment with recombinant human GH (rhGH) has been of interest from several aspects. HIV wasting was a prominent feature in the pre-HAART era, and wasting was included in 1985 as an AIDS-defining condition characterised by weight loss and diarrhoea (Ho et al., 1985). The pathophysiology included a loss of lean and fat mass due to an impaired nutritional state caused by increased metabolism and malabsorption. The anabolic effect of rhGH has been shown to have beneficial effects on the nitrogen balance in a variety of catabolic conditions (Mulligan et al., 1993). With the introduction of HAART, HIV wasting merely disappeared as an entity in the clinic picture; however, the definition has been revised and broadened in the post-HAART era (Moyle et al., 2004), and the discussion of the similarities and dis-similarities between wasting and lipodystrophy has provided new insight in the pathophysiology of these conditions. Despite the indisputable success of HAART in controlling viraemia and reducing mortality and morbidity, some patients face treatment failure and, consequently, CD4 T-cells are depleted and AIDS subsequently develops (Paredes R et al., 2000). In addition, although the immune recovery of the HAART regimen increases the number of mature CD4 cells in a substantial portion of patients, a minority of patients respond to HAART with only a limited increase in CD4 cells (Teixeira et al., 2001). Furthermore, the increased CD4 count is primarily based on a proliferation of peripheral memory T cells and not the whole T cell repertoire, including a central expansion of the naive T cells in the thymus (Teixeira et al., 2001). These immunological shortcomings may be overcome by the thymopoietic effects of rhGH, as shown in both mice and humans, causing the atrophic thymus gland in adults to increase in size and a regeneration of naive T cells (Pires et al., 2004). Immunomodulators, such as GH, can mediate the cross-talk between the neuroendocrine and immune systems.

In normal individuals, GH secretion is regulated in a pulsatile pattern with more than two-thirds secreted during the night (Jørgensen et al., 1994). Growth hormone releasing hormone and somatostatin regulate GH secretion from the pituitary gland; however, GH secretion in extrapituitary tissues is more complicated and may largely depend on local factors, including the cytokine milieu (Clark, 1997). The widespread appearance of GH receptor, a member of the receptor family also known as helix bundle peptide cytokine receptors, are expressed in AT and haemopoietic stem cells, making the actions of GH rather complicated to interpret in advance. In addition, many of the effects of GH are not due to the direct effect of GH through GH receptor signalling, but indirectly mediated through the action of IGF-1, an anabolic hormone produced in the liver.

2. AIMS AND METHODS

When the cohorte studies and the pilot study behind this thesis began, there was no established definition of HALS and no trials had examined low dose GH therapy injected during the daytime in an HIV-positive population. Also at that time, whether HALS was due alone to the HAART was controversial. Moreover, limited data was available on the association of HALS and IR and, as such, no data indicated that the virus and inflammation plaid a role in the pathogenesis of HALS. Thus, the overall purpose of this thesis work was to design a study with emphasis on the metabolic conditions and implications of underlying chronic systemic low-grade inflammation, and a GH study aming to clarify the pathogenesis of and potential therapies for lipodystrophy syndrome in HIV-infected individuals.

2.1. AIMS

The specific major aims were:
1. To test insulin action and β-cell function in patients with HALS.
2. To test whether circulating sex hormones and gene expression in adipose tissue is affected in patients with HALS.
3. To examine insulin resistance and lipid oxidation in patients with HALS.
4. To explore low-grade inflammation in patients with HALS.
5. To examine GH sensitivity in patients with HALS.
6. To examine the effect of low dose GH therapy in patients with HALS.
7. To examine whether long-term treatment with rhGH affects GH factors, HALS, and the immune system.

2.2. METHODS

Subjects

For all studies in the thesis patients were recruited consecutively from the outpatient clinic at the Department of Infectious Diseases, Copenhagen University Hospital, Hvidovre, Denmark. Male patients older than 18 years of age with a positive HIV-1 antibody test and receiving more than 12 months of HAART who complained of changes in fat distribution were included in the studies. The patients were asked to fill out a questionnaire that included nine criteria for lipodystrophy: loss of fat in the face, arms, legs, and buttocks, gain of fat in the abdomen and trunk, more exposed veins, fat pads in the neck region, and lipomas. Further a trained physician performed all of the physical examinations (OA): examination for lipoatrophy in the face, extremities, and buttocks; abdominal obesity; buffalo hump; and lipomas. The patient had to report at least one criterion of lipodystrophy and present at least one of the six signs of lipodystrophy in order to be categorised as an HALS patient. Exclusion criteria were diabetes mellitus, chronic disease other than HIV, an AIDS-related episode or acute infection within the last 3 months, weight loss or gain of more than 4 kg within 6 months (Mulligan et al., 1997), and treatment with antilipid or antidiabetic drugs. None of the subjects were engaged in competitive sports or treated with drugs known to influence glucose metabolism, other than HAART.

Body composition and anthropometry

Dual energy X-ray absorptiometry (DEXA) was performed with a Norland Medical system (XR-36; Fort Atkinson, WI, USA). DEXA scans had a precision of 1% for free fat mass, 3% for total fat mass, 4% for trunk fat mass, and 5% for extremity fat mass. A whole-body scan was performed to estimate the amount of fat in the trunk and extremities. The trunk was defined as the region including the chest, abdomen, and pelvis, excluding the neck and head. The proximal limitations of the leg regions were placed through the hip joints at an angle of approximately 45 degrees, and vertically through the shoulder joints for the arm regions. Two regions of interest were defined to measure the abdominal tissue distribution as previously described (Rosenfalck et al., 1996; Svendsen et al., 1993). The peripheral fat mass was defined as the sum of the arm and leg fat masses. A single-slice CT scan was performed at the Lumbar fourth level to estimate the visceral and subcutaneous AT area. Waist circumference was measured at the level of the umbilicus while the subject was standing and after normal expiration. Hip circumference was measured in the horizontal plane at the level of the maximal extension of the buttocks. Weight, height, waist circumference, and hip circumference were measured in duplicate by the same investigator, and mean values were used.

Experimental conditions

Patients were advised to not alter their normal diet and to not perform strenuous physical exercise for 3 days before metabolic assessments. The subjects were studied in the supine position at room temperature (25°C). A catheter was inserted into the antecubital vein of each arm. One catheter was used for sampling and the other catheter for infusion. After obtaining basal blood samples, indirect calorimetry lasting 30 min was performed to obtain basal glucose oxidation measurements. To characterise the first phase insulin response, a 30-min frequently sampled intravenous glucose tolerance test (FSIGT) was carried out. A biopsy of the AT was subsequently taken from the subcutaneous abdominal region

(periumbilically) by needle. The procedure was performed under local anaesthetic (lidocaine, 5 mg/mL). The tissue was washed thoroughly with isotonic saline, snap frozen in liquid nitrogen, and kept at –80°C for later RNA extraction. In addition, a needle biopsy was taken from the vastus lateralis for analysis of the mitochondrial DNA content. The FSIGT and needle biopsy was followed by hyperinsulinaemic euglycaemic clamp (120 min). During the last 30 min of the clamp period (the insulin-stimulated steady state period), indirect calorimetry was performed again to measure insulin-stimulated glucose oxidation.

FSIGTT and hyperinsulinaemic euglycaemic clamp technique

Baseline blood samples were drawn at -4 min, -2 min, and 0 min. At 0 min, a bolus of 300 mg 20% glucose/kg body weight was injected intravenously over 60 sec. Venous blood was sampled at 2, 4, 6, 8, 10, 15, 20, and 30 min to measure glucose, insulin, and C-peptide. Immediately following the last FSIGT sampling, we started insulin infusion (Actrapid; Novo Nordisk A/S, Bagsvaerd, Denmark) by performing squared priming (0 to +9 min) with a stepwise decline in the insulin infusion rate every third minute, reducing the insulin infusion rate from 100 to 80 to 60 to 40 mU/m² x min. From minute 9 to 120, the insulin infusion rate was fixed at 40 mU/(m² x min). The plasma glucose concentration was maintained constantly as euglycaemia (5 mM) using a variable glucose infusion of 20% glucose. During the clamp, glucose levels were monitored every 5 min and the insulin levels assessed every 15 min. The average glucose infusion rate measured during the last 30 min of the clamp was considered to represent whole body glucose disposal, a marker of peripheral insulin action.

Indirect calorimetry

A ventilated canopy was placed over the patient's head (Deltatrac II Metabolic Monitor; Datex, Helsinki, Finland) and the continuous gas exchange determined. Inspired and expired air was analysed for oxygen concentration using a paramagnetic differential oxygen sensor, and for carbon dioxide using an infrared carbon dioxide sensor. Oxygen consumption and carbon dioxide production were recorded and calculated each minute. After an equilibrium period of 10 min, the average gas exchange over two 30-min steady state periods (basal and insulin-stimulated) was used to calculate the rate of glucose oxidation (Frayn, 1983). Glucose turnover rates were expressed in mg/(min×kg) of free fat mass and presented as mean values for the 30-min steady-state period.

Oral glucose tolerance test

The patients were admitted to Clinical Research Centre, Hvidovre following an 18 h abstinence from HIV medication and an overnight 12 h fast. A catheter was inserted into an antecubital vein. A standard OGTT with 75 g glucose was performed. Blood samples were drawn at -10, 0, 10, 20, 30, 45, 60, 75, 90, 105, and 120 min to measure plasma concentrations of glucose, C-peptide, and insulin. The plasma concentration of FFA was measured at 0, 30, 60, 90, and 120 min. Blood samples were immediately centrifuged at 4°C, and plasma was separated and stored at -80°C for later analysis with the exception of plasma glucose concentrations, which were measured immediately. Patients with a 2 h plasma glucose ≥7.8 mmol/L and <11.1 mmol/L were categorised as having Impaired GT; those with a 2 h plasma glucose <7.8 mmol/L were categorised as having normal GT; and those with a 2 h plasma glucose ≥11.1 mmol/L were categorised as having diabetes mellitus. In six patients, plasma suPAR was measured at 0, 10, 30, 60, 90,

120, and 180 min after glucose ingestion to investigate the post-glucose stability of plasma suPAR.

Assays and biochemistry
Whole blood glucose levels were determined pairwise on two calibrated HemoCue B glucose analysers (HemoCue AB, Sweden) with inter-analyser CV of 3.3%. Plasma glucose was calculated using Fogh-Andersen's equations (Fogh-Andersen and D'Orazio, 1998). Blood samples for plasma insulin and C-peptide determinations were centrifuged immediately at 4°C and stored at -80°C for later analysis. Plasma insulin and C-peptide concentrations were determined using the 1235 AutoDELPHIA™ automatic immunoassay system (Wallac Oy, Turku, Finland). The insulin assay had a detection limit of 3 pM. Cross-reactivity with intact proinsulin was 0.1%, 0.4% with 32-33 split proinsulin, and 66% with 64-65 split proinsulin. The inter-assay CV was 7%. The detection limit of the C-peptide assay was 5 pM. Cross-reactivity with intact proinsulin was 51%, 35% with 32-33 split proinsulin, and 92% with 64-65 split proinsulin. No detectable cross-reactivity with insulin was present. The inter-assay variation was 8%. Plasma FFA were determined using an enzymatic colorimetric method (Wako C test kit; Wako Chemicals GmbH, Germany) with an inter-assay CV of 5%. Total serum cholesterol, high density lipoprotein (HDL) cholesterol, and serum triglycerides were determined by reflection photometry (Ortho-Clinical Diagnostics, NJ, USA) with an inter-assay CV of 2%, 8%, and 2.5%, respectively. Serum cortisol was determined using the radioimmunoassay (RIA) method (Diagnostic System Laboratories, Inc., TX, USA) with an inter-assay CV of 9%. The CD4 count was determined by flow cytometry (FACscan; Becton-Dickinson, NJ, USA) with an inter-assay CV of 7%, and viral load was determined using Roche Amplicor and Roche amplicor Ultrasensitive assay with a detection limit of 20 copies/mL plasma (Roche, Basel, Switzerland), which met the requirements of inter-laboratory quality control.

Androgens, oestrogens, and sex hormone-binding globulin (SHBG) were analysed as described (Lykkesfeldt et al., 1985). Free testosterone was calculated as described by Bartsch (Bartsch, 1980). The measurement of the percentage of free oestradiol was carried out using the centrifugal-ultrafiltration dialysis method (Hammond et al., 1980). The inter-assay CV for were as follows: SHBG, 7.5%; testosterone, 13.8%; free testosterone, 6.4%; dihydrotestosterone, 11.0%; a-4-androstendione, 11.4%; 17β-estradiol, 10.5%; free 17β-estradiol, 10.5%; oestrone, 9.6%; oestrone sulphate, 10.5%; DHEAS, 11.5%. Serum cortisol was determined by RIA (Diagnostic System Laboratories, Houston, TX) with an inter-assay CV of 9%.

The expression of the oestrogen receptor-α (ER-α), α2A-adrenergic-receptor, β-2 adrenergic-receptor, aromatase, Hormone Sensitive Lipase (HSL), LipoProtein Lipase (LPL) , and 11β-hydroxysteroid dehydrogenase type 1 genes was analysed as descriebed in detail in (Paper III: Andersen et al., 2007).

suPAR ELISA
Maxisorp ELISA plates (Nunc, Roskilde, Denmark) were coated with a monoclonal rat anti-human suPAR antibody (VG-1; Viro-Gates SA, Cape Town, South Africa) (3 µg/mL, 100 µL/well) overnight at 4°C. Plates were blocked with phosphate buffered saline (PBS) buffer plus 1% bovine serum albumen and 0.1% Tween-20 for 1 h at room temperature, and then washed three times with PBS buffer containing 0.1% Tween-20. The following reagents

were added to the ELISA plate in duplicate wells: 85 µL dilution buffer (100 mM phosphate, 97.5 mM NaCl, 10 g/L bovine serum albumen Fraction V; Boehringer Mannheim, Penzberg, Germany), 50 U/mL heparin sodium salt (Sigma Chemical Co., St. Louis, MO, USA), 0.1% (v/v) Tween-20 (pH 7.4) containing 1.5 µg/mL mouse anti-human suPAR– horseradish peroxidase (HRP)-labelled antibody (VG-2-HRP, ViroGates SA), and 15 µL of the plasma sample. After 1 h of incubation at 37°C, plates were washed 10 times with PBS buffer plus 0.1% Tween-20, and 100 µL of HRP substrate was added per well (Substrate reagent pack; R&D Systems, Minneapolis, Minnesota, USA). The colour reaction was stopped after 30 min by adding 50 µL/well of 1M H_2SO_4, and the measurements were obtained at 450 nm. The inter-assay variation was 12.4%.

Serum GH levels were determined by an immunofluorometric assay (Delfia, Wallac Oy, Turku, Finland). Serum GH binding protein reduces the GH estimates in GH immunometric assays such as this, but the interference is overcome when incubation is prolonged from 2 h to 24 h. Growth hormone binding protein was determined by immunofunctional time-resolved fluoroimmunoassay as described (Fisker et al., 1996).

Total serum IGF-I and IGF-II were measured by an in-house non-competitive, time-resolved immunofluorometric assay after acid ethanol extraction of the serum as described (Frystyk et al., 1995). Serum free IGF-I and free IGF-II were measured by ultrafiltration as previously described (Frystyk et al., 1994). The serum concentrations of insulin-like growth factor binding protein (IGFBP)-1 and IGFBP-3 were measured by immunoradiometric assay (IRMA; Diagnostic Systems Laboratories, Inc., Webster, TX, USA). The ^{125}I-IGFBP-3 degradation assay was performed as previously described (Lamson et al., 1991). The inter assay CV of control samples averaged 10%.

Calculations
The relative amount of peripheral fat compared to trunk fat, the percentage of limb fat, was calculated (Mynarcik et al., 2000) (i.e. peripheral fat mass/[peripheral fat mass + trunk fat mass] ×100%). The regional fat mass was normalised by body weight (e.g., leg fat (%) = leg fat mass/BW x 100%). The insulin sensitivity index (Si) was calculated as the mean glucose infusion rate during the clamp steady state period divided by the clamp steady state plasma insulin, the clamp steady state glucose concentration, and the body weight or free fat mass. The iv-GT during the FSIGT (Kg) was defined as the slope of the glucose curve between 8 min and 30 min.

As a measure of β-cell function, the acute insulin (first phase) response to glucose ($AIRg_{0-10}$) was calculated using the trapezoidal rule, as the total incremental area under the curve (AUC), 0-10 min after the bolus injection of glucose. In subjects with normal GT, the $AIRg_{0-10}$ will increase as the Si is reduced. The product of these two parameters is approximately a constant, termed the disposition index (DI = Si × $AIRg_{0-10}$). Thus, as suggested by Bergman (Bergman et al., 1981) and confirmed by Kahn (Kahn et al., 1993), the relationship between Si and $AIRg_{0-10}$ is a hyperbola. Therefore, a failure to fit a hyperbolic relationship between Si and $AIRg_{0-10}$ might be due to insufficient adaptation of the pancreatic β-cells to the concomitant insulin sensitivity. The following strategy was designed to identify patients with a reduced capacity for β-cell adaptation. After sorting the patients according to increasing disposition index (=Si x $AIRg_{0-10}$), we analysed whether the

omission of those with the lowest disposition index lead to an improved fit of the hyperbolic relationship between Si and $AIRg_{0-10}$, such that it became statistically significant and significantly better than the fit with all patients. In line with the work of Kahn (Kahn et al., 1993), we also calculated the relationship between fasting insulin and Si, testing whether this relationship fits a hyperbola.

Pre-hepatic insulin secretion was calculated from plasma C-peptide measurements using the ISEC (Insulin SECretion) computer program (Hovorka et al., 1996). The model is based on the assumptions that insulin and C-peptide are co-secreted by the pancreas in an equimolar fashion and that the liver does not clear C-peptide. ISEC has been validated for calculating pre-hepatic insulin secretion during the FSIGT (Hovorka et al., 1998; Kjems et al., 2001) and has been applied to calculate pre-hepatic insulin secretion profiles during meal tolerance tests, hyperinsulinaemic euglycaemic clamp, and under basal conditions. The calculated pre-hepatic insulin response to iv glucose for each study group revealed that practically all insulin was secreted within the first 6 min. We denoted this amount of insulin, the acute pre-hepatic first phase insulin secretory response ($AIRs_{0-6}$), which was calculated as the AUC 0-6 min after the bolus injection of glucose. $AIRs_{0-6}$ was expressed as $pmol \times kg^{-1}$ and for 'whole body' response as pmol.

We calculated the insulin Si suggested by Matsuda and DeFronzo for the OGTT, denoted $ISI_{composite}$ (Matsuda and DeFronzo, 1999):

$$ISI_{composite} = \frac{10,000}{\sqrt{FPG \cdot FPI \cdot MG_{0-120} \cdot MI_{0-120}}}$$

where FPG and FPI are the fasting plasma glucose and insulin concentrations, respectively. MG_{0-120} and MI_{0-120} are the means of the glucose and insulin concentrations, respectively, measured at 0, 30, 60, 90, and 120 min during the OGTT. The concentration of glucose is expressed in mg/dL and the insulin concentration in μU/mL. The unit of $ISI_{composite}$ is $L^2 \times mg^{-1} \times μU^{-1} \times ISI_{composite}$ and has been shown to correlate closely with the M-value of the glucose clamp in individuals who display a range of GT from normal to diabetes (Matsuda and DeFronzo, 1999).

3. RESULTS AND DISCUSSION

3.1. GLUCOSE METABOLISM AND INSULIN RESISTANCE

In the pre-HAART era, glucose measurements were not a major concern apart from the iatrogen-induced hyperglycaemia caused by corticosteroid or pentamidine therapy (Assan et al., 1995). HIV infection *per se* did not, under normal immunological conditions, induce IR (Hommes et al., 1991). In the HAART era, the first reports indicating a higher prevalence of IR and glucose intolerance associated it with HIV protease inhibitor (PI) treatment, which arose in the mid-90s ("Archived - Reports of Diabetes and Hyperglycemia in Patients Receiving Protease Inhibitors for the Treatment of Human Immunodeficiency Virus (HIV)," 1997). These reports were soon followed by a range of papers demonstrating impaired GT in 35% of patients who had bloodsucker above the normal range (Behrens et al., 1999; Hadigan et al., 2001).

Several groups, including our own, have studied the pathophysiology of IR in HALS. In 1999, Vigouroux assessed for the first time

GT, insulin sensitivity, and lipid parameters in a group of HIV-infected patients with HALS. All patients received HAART including PIs. Fourteen patients with marked facial lipoatrophy were evaluated by an OGTT and compared with 20 non-HALS PI-treated patients. The measurements indicated that HALS was associated with IR and hypertriglyceridaemia (Vigouroux et al., 1999). Glucose metabolism in HALS was further characterised comprehensively in 2001 by assessing glucose disposal and its pathways, glucose production, and plasma FFA levels in six HIV-positive patients with HALS compared to six healthy individuals. The data showed that post-absorptive insulin concentration and glucose production was 47% higher in HALS patients compared to controls. The authors concluded that post-absorptive glucose production is increased in HIV-1-infected patients with HALS. Moreover, the ability of insulin to suppress both endogenous glucose production and lipolysis and to stimulate peripheral glucose uptake and its metabolic pathways was reduced (van der Valk et al., 2001).

In 2003 we investigated 18 males with HALS and 18 HIV-positive males without HALS. The duration and modality of antiretroviral therapy was similar between study groups. A hyperinsulinaemic euglycaemic clamp revealed an impaired glucose disposal rate in HALS patients. By indirect calorimetry, HALS patients showed impaired non-oxidative glucose metabolism (NOGM), whereas the levels of basal and insulin-stimulated oxidative glucose metabolism were not significantly different between groups. Despite comparable total fat masses, DEXA revealed that the percentage of limb fat was reduced significantly in HALS patients. Linear regression analysis indicated that the percentage of limb fat explained 53% of the variability of GDR and 45% of the variability of NOGM in HALS patients. In HALS patients, leg fat mass positively correlated with NOGM, whereas the abdominal fat mass and NOGM did not correlate. Analysing the relationship between first phase insulin secretion and insulin sensitivity, the HALS patients exhibited impaired insulin secretion. The data suggest that fat redistribution, independent of antiretroviral therapy, is highly related to IR in HALS patients. Furthermore, in HALS patients, impaired glucose metabolism most likely relates to decreased NOGM and defects in β-cell function (Paper 1: Ove Andersen et al., 2003).

Further, we investigated whether the incretin hormones (GLP-1 and GIP) contribute to impaired GT among HIV-infected patients on HAART. We included 18 HIV-infected male patients with normal GT and compared them with 10 HIV-infected male patients with impaired GT. The data demonstrated that the AUC for GLP-1 was increased by 250% in impaired GT patients compared to normal GT patients, whereas the AUC for GIP did not differ significantly between the study groups. These data suggest that glucose-intolerant, HIV-infected male patient's exhibits enhanced GLP-1 responses to oral glucose compared to normal GT HIV-infected male patients. This finding may indicate a compensatory mechanism rather than explain impaired GT (Paper II: Andersen et al., 2005). These data makes it more unlikely that the GLP-1 analogues will be of any benefit to HALS patients, in contrast to their expected potential role in type-2 diabetes and metabolic syndrome in HIV-negative patients (Chyan and Chuang, 2007).

In skeletal muscle biopsies, we found that the impaired NOGM correlated with impaired glycogen synthase activity. The defective glycogen synthase activity was due to specific defects in insulin signalling downstream of PI3-K at the level of Akt. The basal activity of insulin receptor substrate-1-associated PI3-K tended to be

increased in HALS patients, and the insulin stimulation significantly increased the PI3-K activity in HALS and non-HALS HAART-treated patients, suggesting a mechanism for the IR (Haugaard et al., 2005a). In concordance with these observations there was later presented data from a placebo controlled crossover study with acipimox (Lindegaard et al., 2007). The data showed that suppression of lipolysis improved insulin-stimulated peripheral glucose-uptake in nine patients with HALS. The increased glucose-uptake may be explained, in part, by increased dephosphorylation of glycogen synthase, resulting in increased glycogen synthase activity in skeletal muscle.

The role of PIs in the pathogenesis of IR and HALS has been examined in numerous in vitro and clinical studies. The PIs indinavir, saquinavir, ritonavir, atazanavir/ritonavir, and loinavir/ ritonavir have been shown to affect more proximal steps of insulin signalling involving insulin receptor binding and glucose transporter 4 activity (Flint et al., 2009; Hertel et al., 2004; Noor et al., 2006). Therefore, HALS patients seem to be prone to "dual detrimental action" upon insulin signalling. HALS could account for more distal defect, and PIs for more proximal defect, mechanisms. Together, these actions may create a vicious cycle that greatly enhances the risk of type 2 diabetes.

The mechanism of mitochondrial depletion leading to hyperlactataemia caused by dysfunction of the oxidative part of the respiratory chain has been examined thoroughly in in vitro studies as well as clinical trials (Ribera et al., 2008). All studies have been based upon the hypothesis that NRTI inhibits mitochondrial replication in adipocytes by inhibiting the enzyme polymerase gamma, which is responsible for the replication of mitochondrial DNA (Brinkman, 2001; Brinkman et al., 1998). The prevalence of hyperlactataemia, which has been shown to be up to 9%, in cohort studies (Moyle et al., 2002) may have been overestimated; we demonstrated that an unintended postponement of blood samples at room temperature accelerates the glycolytic processes and significantly increases p-lactate (O. Andersen et al., 2003). The data was later confirmed (Dubé et al., 2005) and, routine testing of HIV-positive asymptomatic patients cannot be recommended (Moyle et al., 2002). The clinical significance of mitochondrial toxicity, particularly that due to thymidine analogues such as stavudine, is decreasing due to diminished use of these drugs in the developed world, supported by results, including our own (Haugaard et al., 2005b), indicating that the depletion of mithochondrial DNA has to reach a threshold lower than that observed in HIV-positive patients on HAART (Brinkman et al., 1999; Cossarizza et al., 2002; McComsey et al., 2002)

In vitro, the pyrimidine precursor uridine abrogates thymidine analogue-induced toxicity in adipocytes (Walker and Venhoff, 2005). A randomised, double-blind, placebo-controlled trial with 20 HALS patients found that the proportion of limb fat to total fat increased from 18% to 25% (P<0.05) in the uridine group. Liver fat content and lean body mass remained unchanged in both groups. High density lipoprotein-cholesterol concentrations decreased in the uridine group and increased in the placebo group, whereas fasting serum insulin concentrations did not change (Sutinen et al., 2007). These findings were supported in another study of 16 HALS patients on stavudine-containing antiretroviral therapy, who received NucleomaxX, a dietary supplement with a high bioavailability of uridine. The body mass index, lactate, lipids, insulin, and

homeostasis model assessment of IR were unaltered. Fat and peripheral blood and mononuclear cell mithochondrial DNA levels did not correlate with each other and exhibited no changes throughout the study. Lipoatrophy scores by patients and physician improved significantly compared to the scores at study entry (McComsey et al., 2008).

3.2. SEX HORMONES

In many conditions that affect sex hormone binding protein (SHBG) concentrations, such as obesity, type 2 diabetes, ageing and HIV-infection, total testosterone concentrations are altered because of changes in SHBG concentrations; in these conditions, expert panels have recommended the determination of free testosterone (FT) concentration to obtain an accurate assessment of androgen status (Zakharov et al., 2015). Low levels of testosterone have been found in HIV-positive men (Kopicko et al., 1999). Low testosterone can lead to depression, fatigue, low libido, and a decrease in lean mass. Reports of low testosterone levels were common in the pre-HAART era. In the HAART era, links between different classes of anti-HIV drugs and specific hormone levels have been demonstrated (Hadigan et al., 2000). Anti-HIV therapy seems to increase the levels of testosterone and 17β-estradiol. Protease inhibitors were specifically linked to increased levels of testosterone, and non-nucleoside analogues were linked to increased levels of 17β-estradiol (Collazos et al., 2002). However, the mechanism is poorly understood. One possibility is that PIs, and non-nucleoside analogues, can impair the catabolism of sex hormones by the cytochrome P450 system in the liver, increasing their levels in the blood (Collazos et al., 2002; Inaba et al., 1997).

Circulating oestradiol and testosterone have been shown to be increased in HIV-infected patients following HAART and may influence fat distribution and insulin sensitivity (Collazos et al., 2002). Oestradiol increases subcutaneous AT in humans, possibly through binding oestrogen receptor-α, which in turn activates anti-lipolytic α2A-adrenergic receptor (Pedersen et al., 2001).

We addressed these issues by examining 31 HIV-positive patients for circulating pituitary-gonadal-axis hormones and the expression of receptor genes in subcutaneous adipose. We were able to demonstrate that total and free oestradiol and testosterone are decreased in HALS compared to non-HALS (Paper III: Andersen et al., 2007). Free testosterone was calculated as described by Bartsch (Bartsch, 1980), however a new paper from Zakharov has suggested (Zakharov et al., 2015) that the fraction of free circulating testosterone, is substantially greater than generally assumed. In contrast, luteinizing hormone, follicle-stimulating hormone, and prolactin were similar and normal in both study groups. The ratio of subcutaneous to total abdominal fat mass, limb fat, and insulin sensitivity, which were all decreased in HALS, correlated positively with both plasma oestradiol and testosterone. The glycerol concentration during clamp, a biomarker of lipolysis, was negative correlated with α2A-adrenergic receptor, the ratio of subcutaneous to total abdominal fat mass, and limb fat. The α2A-adrenergic receptor correlated positively with oestrogen receptor-α. These results fit the hypothesis that sex hormones play a role in the altered fat distribution and insulin sensitivity of male patients with HIV-associated lipodystrophy. The effect of oestra-

diol on the subcutaneous fat depot and lipolysis may be mediated, in part, through binding to oestrogen receptor-α, activating the anti-lipolytic α2A-adrenergic receptor.

In our HALS patients, testosterone and DHEAS were within the normal range compared to an HIV-negative male population, but lower than the non-HALS HAART-treated control group. This finding might indicate a failure of these precursors to convert to oestradiol as an explanation of the low oestradiol found in HALS. These data are in line with other studies that reported reduced plasma levels of DHEAS in HALS (Paper III: Andersen et al., 2007; Piketty et al., 2001). DHEAS is a ligand for, and has been shown to increase the expression of, PPAR-α in subcutaneous adipocytes and to affect the β-oxidation pathway (Smith and Skelton, 2001).

The association between sex hormone levels and the development of lipoatrophy in HIV-infected men prior and after two years of HAART treatment has also been evaluated. The investigators found hypogonadism in the majority of HIV-infected patients and that lipoatrophy was associated with an increase in luteinizing hormone and a lack of increased dehydroepiandrosterone (DHEA) levels (Chen and Parker, 2004; Wunder et al., 2008). Therefore, we can speculate that DHEAS may have a beneficial effect on HALS. The possible anti-inflammatory effects of DHEA, which is reviewed in (Chen and Parker, 2004) also include inhibition of oxygen radical secretion as well as production of TNF-α, resulting in decreased tissue destruction. Serum DHEA and DHEAS levels have been associated with IL-6 levels and dysregulation of IL-10 production. Other immunomodulatory effects of DHEA could be related to its metabolites, namely androstenedione, testosterone, and oestradiol, but this hypothesis remains to be examined (Labrie et al., 1997; Srinivasan et al., 2010). The results suggest a link between sex hormones and the different physical changes in HALS and have to be examined further in placebo-controlled trials with hormone therapy. How much of these perturbations in sex hormone levels are due to or caused by HALS remain to be elucidated in prospective studies, but the data adds another layer to the complexity of HALS. In addition, oestrogen receptors have been shown to regulate the expression of PPAR-γ co-activator 1, and sex steroids can regulate inflammatory cytokines (Yu and Chaudry, 2009). Thus, modulation of the prevailing sex hormone milieu might be a therapeutic option for improving HALS.

Research that looks carefully at the impact of sex on HIV and the response to therapy is critical to understanding gender differences. Studies of the role of hormonal mechanisms will probably provide information concerning gender-related differences in the development of HALS and steps that can be taken for better treatment and care of both men and women. To achieve this understanding, studies must be designed to enable the analysis of gender impact when the data are evaluated.

3.3. ADIPOSE TISSUE AND LOW-GRADE INFLAMMATION

Over the past few decades, significant advances have been made in delineating key extracellular and intracellular stimulators of fat cell formation, or adipogenesis. Research has focused on finding new specific inhibitors of adipogenesis (Colitti and Grasso, 2014; Harp, 2004). However, understanding the balance between positive and negative regulators of adipogenesis has important health-related implications for HIV-positive patients with HALS. Growth hormone stimulates adipogenesis through activation of

the Stat5A/5B-PPAR-γ pathway. The adipogenic effect of GH was partly caused by a stimulation of the insulin-induced adipogenesis of 3T3-L1 cells with early induction of PPAR-γ-2 expression (Kawai et al., 2007). The lipolytic action is also able to stimulate pre-adipocyte differentiation in vitro (Tominaga et al., 2002). In addition, the autonomous nervous system has a dual effect on adipocyte differentiation with increased lipolysis through β-1 and β-2 receptors and a decrease via α -2 receptors (Lafontan and Berlan, 2003).

Some PIs alter adipokine secretion and lipid content through reactive oxygen species production in human subcutaneous adipocytes (Lagathu et al., 2007).Thymidine analogues alter adipocyte functions, but their effect on adipokine secretion is not reverted by inhibiting reactive oxygen species production. Increased chemokine/cytokine production by adipocytes and macrophages could be involved in macrophage recruitment and participate in lipoatrophy and IR.

Low-grade inflammation
Research of the mechanism underlying IR, insulin signalling and HALS has been influenced by research on the hypothesis that chronic low-grade inflammation is a major mechanistic player in IR (Kolb and Mandrup-Poulsen, 2005), and consistent with the AT damage and remodelling that characterise HALS.

The AT dysfunction observed in HALS can result in alterations in FFA and adipokine release and may underlie the metabolic complications. Several groups have investigated the adipokine system, especially TNF-α. We examined whether plasma TNF-α is associated with the degree to which insulin suppresses markers of lipolysis, such as FFA and the net lipid oxidation rate. The net lipid oxidation rate was estimated by indirect calorimetry during fasting and the hyperinsulinaemic euglycaemic clamp steady state in normoglycaemic HIV-infected men on HAART. In HALS, TNF-α correlated with clamp FFA, clamp net lipid oxidation rate, incremental FFA, and incremental net lipid oxidation rate; all of which, with the exception of the clamp net lipid oxidation rate correlation, remained significant after correction for insulin sensitivity. None of these correlations were significant in non-HALS patients. In all patients, TNF-α correlated with clamp FFA, clamp net lipid oxidation rate, and incremental, with the two former correlations remaining significant after correction for insulin sensitivity. Net lipid oxidation rate and FFA (fasting and clamp values combined) correlated strongly in both HALS and non-HALS. However, fasting FFA and net lipid oxidation rate were not different for HALS patients, and the insulin-mediated suppression of FFA and net lipid oxidation rate was attenuated significantly in HALS patients. Our data indicate that higher TNF-α, independent of insulin sensitivity, is associated with attenuated insulin-mediated suppression of FFA and net lipid oxidation rate in HALS. This sugges that TNF-α stimulates lipolysis in this syndrome. Furthermore, FFA may be a major determinant of net lipid oxidation rate in HIV-infected patients on HAART (Paper IV: Haugaard et al., 2006b).

Adiponectin
We investigated the circulating levels and gene expression of adiponectin in subcutaneous AT from the previous described cohorte study. The implications of cytokines for adiponectin levels were investigated by determining the circulating levels of TNF-α, IL-6, and IL-8, as well as the expression of the genes encoding these cy-

tokines in the AT. HIV-associated lipodystrophy syndrome patients exhibited 40% reduced plasma adiponectin levels compared to non-HALS subjects. Correspondingly, adiponectin mRNA levels in AT were reduced by >50%. HIV-associated lipodystrophy syndrome patients were insulin resistant, and a significant positive correlation was found between plasma adiponectin and insulin sensitivity and percent limb fat. The TNF-α, IL-6, and IL-8 mRNA was increased significantly in the AT of HALS subjects, and both TNF-α mRNA and plasma TNF-α were negatively correlated to plasma adiponectin in AT. Finally, TNF- α was found to inhibit human AT adiponectin mRNA by 80% in vitro. Furthermore, HALS patients have reduced levels of plasma adiponectin and adiponectin mRNA in AT.

Increased cytokine mRNA in AT is hypothesized to exert an inhibitory effect on adiponectin gene expression and, consequently, to play a role in the reduced plasma adiponectin levels found in HALS patients. Similar findings have been reported in 2014 by Freits and colleagues (Paula Freitas, 2014). Given the low levels of adiponectin in the metabolic syndrome and in subjects with HALS, and the beneficial effect of the adipokine in animal studies, there is exciting potential for adiponectin replacement therapy in IR and related disorders (Whitehead et al., 2006). Furthermore, adiponectin secretion from adipocytes is enhanced by thiazolidinediones, which are also known to antagonise the effects of TNF-α. Thus, adiponectin may be one common mechanism by which TNF-α promotes, and the thiazolidinediones suppress, IR and inflammation. In contrast to expectations, a small placebo-controlled study showed that an increase in adiponectin induced by rosiglitazone did not improve glucose metabolism in HIV-infected patients with overt lipoatrophy. If these findings can be confirmed by larger studies, they could question the importance of adiponectin in regulating glucose metabolism in HALS (Blümer et al., 2009; Savage and O'Rahilly, 2010).

Leptin
Mounting evidence reveals that adiponectin plays a positive role in control of obesity, whereas abnormal expression/secretion of leptin is linked to obesity and obesity-related disease in general (Havel, 2002). However the concern that rosiglitazone increases the cardiovascular risk (Psaty and Furberg, 2007) has hamered its clinical use and a meta-analysis of six placebo-controlled trials of thiazolidinedione therapy for HIV lipoatrophy has shown more promising results for pioglitazone (Raboud et al., 2010)
Leptin deficiency is associated with dyslipidaemia and IR in animals and humans with lipoatrophy, and leptin replacement may ameliorate these abnormalities (Savage and O'Rahilly, 2010). A 2-month double-blind, placebo-controlled crossover clinical study with 0.04 mg/kg/d of recombinant human leptin (rhLeptin) showed a 15% decrease in truncal fat mass and an improvement in inflammatory markers and insulin sensitivity (Lee et al., 2006). These data have been confirmed and extended in a 6-month open-label pilot study with eight lipoatrophic HIV-infected patients with rhLeptin. Visceral fat decreased significantly by 32% with no changes in peripheral fat, fasting insulin decreased, and endogenous glucose production decreased during fasting and hyperinsulinaemia, providing evidence of improved hepatic insulin sensitivity. Significant decreases were found in fasting total, direct LDL, and non-HDL cholesterol. The HDL cholesterol increased. Triglycerides, whole-body lipolysis, and FFAs decreased during fasting and hyperinsulinaemia. The rhLeptin was well tolerated but decreased lean mass. However recently, it has been shown that

upto 20-week treatment with leptin in 13 patients with congenital or acquired lipodystrophy did not significantly affect insulin secretion or β-cell function (Muniyappa et al., 2014). The role of adipokines (i.e. adiponectin and leptin) in region-specific alterations between subcutaneous and omental adipocytes and apoptosis has shown that ritonavir, but not atazanavir, exposure can inhibit the differentiation of subcutaneous and omental adipocytes to a similar extent in HALS (Jones et al., 2008). Furthermore, mounting evidence reveals that abnormal expression/secretion of leptin is linked to obesity and obesity-related disease, whereas adiponectin plays a positive role in control of obesity.

PAI-1
We, among others, have investigated the association between circulating levels of PAI-1 and locally produced PAI-1 in the AT of HIV-positive individuals with and without HALS. We found that plasma PAI-1 was increased in HALS compared to non-HALS patients and positively correlated with BMI, plasma TNF-α, soluble tumour necrosis factor receptor (sTNFR)I, sTNFRII, and visceral fat. Moreover, plasma PAI-1 was negatively associated with insulin sensitivity and the percentage of limb fat. A positive correlation was found between plasma PAI-1, TNF-α mRNA, and IL-8 mRNA levels in AT. However, we found no association between plasma PAI-1 and the PAI-1 mRNA level in AT. We concluded that plasma PAI-1 is increased in HIV-associated lipodystrophy, together with decreased insulin sensitivity, enhanced AT expression of cytokines (Il-6, Il-8, and TNF-α), and elevated plasma levels of sTNFRI. These data support the hypothesis that a dysregulation of TNF-α may play a role in the enhanced PAI-1 in HALS, and that enhanced PAI-1 may increase the cardiovascular risk of these HALS patients (He et al., 2005).

Biologics that antagonise the biological activity of TNF-α (e.g., infliximab, etanercept, and adalimumab) are increasingly used for treating immune-mediated inflammatory diseases worldwide. A few studies have examined the effect of these drugs on glucose metabolism with conflicting results (Kiortsis et al., 2005; Rosenvinge et al., 2007). However, TNF-α antagonists are known to increase the risk of reactivation and infection, particularly intracellular bacteria such as *Mycobacterium tuberculosis,* and patients with viral infections have to be carefully monitored. In fact, anti-TNF-α treatment in rheumatoid arthritis decreases the pro-coagulant and fibrinolytic activity with a significant decrease in PAI-1 antigen level (Agirbasli et al., 2006).

suPAR
Soluble urokinase plasminogen activator receptor has been shown to reflect the immunological and pro-inflammatory status of the HIV-infected patient. HAART reduces suPAR independent of the immunological response to HAART (Jaffar et al., 2005). Though, suPAR remains elevated in some HIV-infected patients, possibly reflecting a low-grade pro-inflammatory state. We examined the concentration of suPAR and the possible association with the metabolic status of HIV-infected patients on HAART.

Fasting plasma suPAR was analysed in the 36 normoglycaemic HIV-infected patients on HAART who had determined their, estimated insulin sensitivity and NOGM by euglycaemic hyperinsulinaemic clamp combined with indirect calorimetry and glucose tracer infusion. Plasma suPAR and non-HDL-cholesterol levels were increased, and Rd, NOGM, and limb fat were decreased, in

HALS patients compared to non-HALS. Soluble urokinase plasminogen activator receptor correlated positively with non-HDL-cholesterol and inversely with Rd, NOGM, and limb fat. Soluble urokinase plasminogen activator receptor also correlated positively with lymphocyte count and TNF-α, but not with IL-6. Soluble urokinase plasminogen activator receptor was a stronger predictor of dysmetabolism than TNF-α and IL-6 (Paper VI: Andersen et al., 2008a).

We extended these findings by investigating the association of suPAR with glucose metabolic insufficiency during an OGTT. In 16 HIV-infected patients with HALS and 15 HIV-infected patients without HALS, GT, insulin sensitivity (ISIcomposite), pre-hepatic insulin secretion, proinsulin level, and the suppression of FFA were determined. HIV-associated lipodystrophy syndrome was associated with a 70% increase in plasma suPAR. During the OGTT, plasma suPAR correlated inversely with ISIcomposite and positively with the 2 h plasma glucose, fasting insulin secretion, fasting intact proinsulin, and FFA level. Soluble urokinase plasminogen activator receptor remained a significant marker of GT and insulin sensitivity after adjusting for anthropometric measures (BMI, limb adiposity, and fat mass), immune markers (CD4, HIV RNA, duration of HIV infection), dyslipidaemia (plasma total cholesterol, triglycerides, and FFA level during the OGTT) (Paper V: Andersen et al., 2008b). These data support the hypothesis that suPAR participates in different aspects of alterations in glucose metabolism. How this action is mediated through an interaction with PI3-K and the insulinsignalling cascade, remains to be determined. Indeed, it is still under debate how suPAR may reflect the metabolic status of the HIV-infected patient, linking low-grade inflammation, immune constitution, lipid and glucose metabolism, and fat redistribution together in an HIV-associated inflammatory milieu.

In a cross-sectional study of 1142 HIV-infected patients, we have extended our findings and explored how the levels of suPAR is associated to riskfactors of dysmetabolism and markers of biological ageing in HIV-infected patients (Langkilde et al., 2012)("Correction," 2014). We found elevated suPAR levels in untreated patients compared to patients on cART. Moreover, we observed a significant positive association between suPAR and HIV RNA levels in cART-treated patients. Age, metabolic syndrome and low leg muscle mass all riskfactors of biological ageing, were also significantly associated with high suPAR levels. Our study therefore indicates, that also other aspects of living with HIV than virologic and immunologic markers add to the increased inflammation in HIV-infected patients. In this context it is noatable that skeletal muscle is crucial for maintaining blood glucose control and energy balance. The glucose uptake into muscle is stimulated by contraction or by postprandial insulin secretion and data support that increasing musclemass improve metabolism (McPherron et al., 2013). The great amount of data obtained in HIV infected patients lead us to investigate the effect of suPAR in the general population.

Indeed the inflammatory properties of suPAR and the elevated suPAR levels are associated with systemic inflammation and increased mortality in a number of bacterial (Donadello et al., 2012; Kofoed et al., 2007), viral, and parasitic diseases, as well as in certain types of cancer (de Bock and Wang, 2004; Sier et al., 1998), we investigated the potential value of suPAR as a risk marker for disease and mortality in an observational prospective cohort study, MONICA10. The 2602 participants came from a general Caucasian population, in Copenhagen County, Denmark and were aged 41, 51, 61 or 71 years. The cohort was followed for 12 years. Blood samples were analysed for suPAR levels and risk of cancer (n = 308), CVD (n = 301), type 2 diabetes (n = 59) and mortality (n = 411) was assessed with a multivariate proportional hazards model using Cox regression. We found that elevated suPAR concentrations, at baseline was associated with an increased risk of cancer, CVD, type 2 diabetes and mortality during follow-up and remained significantly associated. (Paper VII: Eugen-Olsen et al., 2010).

3.4. GROWTH HORMONE AND RELATED PROTEINS

The lipolytic effect of GH on visceral AT was shown in an obese HIV-negative population (Johannsson et al., 1997) and several studies in HALS patients with increased visceral abdominal fat (Engelson et al., 2002; Kotler et al., 2004; Lo et al., 2001; Mauss et al., 1999; Wanke et al., 1999). The results of these high dose trials were hampered by a high rate of side effects in regards to glucogenesis and a concern regarding the lipolytic action in the lipoatrophic body areas. However, later reports in HIV-negative young adults and obese men with low dose GH treatment have indicated a different action of GH on glucose metabolism and lipolysis (Yuen and Dunger, 2006).

All trials have injected rhGH in the evening, mimicking the treatment in GH-deficient patients, despite the fact that lipodystrophic HIV patients do not suffer from GH-deficiency but merely resemble patients with the same abdominal size (Heijligenberg et al., 1996). Animal experiments with rats have demonstrated that a low continuous infusion of rhGH results in an increase in IGF-1 to a higher level than the same dose of rhGH given as an normal injection and later it has been demonstrated that continous rather than a single dose treatment with rhIGF-1 in cachectic HIV positive patients have a higher anabolic effect (Lieberman et al., 1994). These observations, together with an unpublished case (personal communication, Johan Iversen), led us to investigate rhGH given as an injection in the middle of the day (1 pm- 3 pm), when the normal secretion of endogenous GH is traditionally at its lowest. The main benefit of this setup is that one achieves the maximum IGF-1 response to the lowest possible rhGH dose, thereby minimising the diabetogenic and lipolytic effect of GH, as IGF-1 does not exhibit the same effect on glucose metabolism or lipolysis as GH (Yuen and Dunger, 2007). In addition, the normal pituitary feedback mechanism of IGF-1 on GH secretion is not comparable when looking at the local production of GH/IGF-1, which interacts with the local cytokine milieu. We characterised the GH secretion pattern of six patients with lipodystrophy and found a normal GH pattern and increased GH sensitivity (Paper VIII: Ove Andersen et al., 2004).

Growth hormone sensitivity
The GH/IGF-1 axis has been extensively studied in healthy populations (Gómez et al., 2003) and various diseases, such as IR (Bereket et al., 1995) and wasting in AIDS patients (Grinspoon et al., 1996). In an attempt to measure GH receptor sensitivity in HIV patients, we measured overnight fasting concentrations of GH binding protein, which has been suggested to be a marker of GH receptor sensitivity (Amit et al., 2000) and GH-sensitive IGF-I and IGFBP-3, in HIV-infected patients with HALS and non-HALS and antiretroviral naive HIV-infected patients.

Three-hour GH suppression tests using oral glucose, reflecting the prandial setting, were performed to determine the dynamics of GH secretion in the daily postprandial period. Total IGF-I and IGFBP-3 were increased in HALS compared to non-HALS but did not differ significantly between non-HALS and antiretroviral naive HIV-infected patients. The AUC for GH during the GH suppression test was decreased in HALS compared to non-HALS. The ratio of limb to trunk fat, which was decreased in HALS compared to non-HALS and antiretroviral naive HIV-infected patients, correlated positively with AUC for GH and rebound-GH. All groups displayed GH suppression during the suppression test, and all groups, except HALS, displayed a rebound of GH, which is likely explained by persistently increased plasma glucose in HALS compared to non-HALS and antiretroviral naive HIV-infected patients. Growth hormone binding protein inversely correlated with AUC for GH. In the blood, 99% of IGF-1 circulates bound to the GH-dependent IGFBP-3 and only a small fraction as bioactive free IGF-1 (Baxter, 1994). Increased IGFBP-3 protease activity may be important for IGF-1 bioactivity (Skjærbæk et al., 1998) and has been demonstrated in type 2 diabetes and GH deficiency (Lassarre et al., 1994). In our studies, no increase of IGFBP-3 protease was found (Paper VIII: Ove Andersen et al., 2004; Haugaard et al., 2004). These results suggest that GH-target tissues are at least as GH-sensitive in HALS as in HIV-infected patients without lipodystrophy. The data further support the hypothesis that HALS patients compensate impaired GH secretion caused by an increased amount of visceral AT by increasing the GH sensitivity of target tissue.

Low-dose growth hormone therapy
Treatment of HALS patients with high doses (2-6 mg/d) of rhGH has been shown to increase concentrations of total IGF-I more than two-fold above the upper normal range and is accompanied by adverse effects, such as joint pain and glucose intolerance (Burgess and Wanke, 2005).

An open-label study was presented in 2001 in which Lo evaluated the effects of a lower pharmacological dose of rhGH (3 mg/d) in eight men with HIV-associated fat accumulation. A significant loss of body fat and gain in lean tissue was reported. However, insulin-mediated glucose disposal transiently decreased after one month of GH therapy (Lo et al., 2001). In addition, the group reported changes in the hepatic carbohydrate and fat metabolism associated with the GH treatment (Schwarz et al., 2002). Hepatic insulin sensitivity was assessed under both fasting and hyperinsulinaemic euglycaemic clamp conditions prior to and after 1 and 6 months of GH treatment in five patients. Fasting endogenous glucose production increased significantly at 1 month, and the increase was sustained at 6 months of GH treatment. This increase in endogenous glucose production was driven, in part, by increased glucogenesis at 1 and 6 months, and small changes in hepatic glycogenolysis also contributed to the increase. A sustained increase in lipolysis and progressive decreases in hepatic fractional de novo lipogenesis and triglyceride concentrations were observed with GH treatment. These changes were accompanied by an improved lipid profile with a significant increase in HDL-cholesterol and significant decreases in total and LDL-cholesterol and triglyceride levels; the latter was consistent with the decrease in hepatic DNL. During hyperinsulinaemic euglycaemic glucose clamp, endogenous glucose production and glucogenesis were markedly suppressed compared to the corresponding time points under fasting conditions, though less so when measured after one

month of GH treatment. Thus, in HIV-infected patients with abnormal fat distribution, pharmacological doses of GH improved the overall lipid profile but worsened glucose homeostasis under both fasting and hyperinsulinaemic conditions. The combined implications of these positive and negative metabolic effects on CVD risk remain unknown, but high dose (3 mg/d) GH cannot be recommended for HALS. Moreover, HALS patients are likely to be GH-sensitive as we have demonstrated in (Paper VIII: Ove Andersen et al., 2004), indicating that lower doses of GH may be a treatment option in these patients.

We therefore performed a 16-week open-label prospective pilot study in six male HALS patients (2 normal-weight patients with normal GT, 2 normal-weight patients with impaired GT, and 2 obese patients with diabetes) using a subcutaneous low dose of hGH (0.7 mg/d), aiming to examine the impact on total and free IGF-I and the fat distribution. Glucose metabolism was examined by OGTT and hyperinsulinaemic euglycaemic clamp. Total IGF-I increased 2-fold and free IGF-I increased 2.5-fold to a level in the upper normal range. Patients reported improvements in their lipodystrophy. Total fat mass, exercise capacity, GT, glucose disposal rate, and immune status did not change during the 16-week treatment. The patients did not complain of arthralgia or other known GH-related side effects (Paper IX O. Andersen et al., 2004). We also evaluated whether the two obese insulin-resistant patients had GH-resistance. The two patients with diabetes displayed an impaired rebound of GH during a 5-h OGTT compared to the normal GT and impaired GT individuals. The IGF-I levels were near lower normal limits in all patients before GH administration. During GH administration, the individuals with diabetes exhibited a 122% and 152% increase in total and free IGF-I, respectively (means of weeks 2, 6, 10, and 16), those with normal GT exhibited 87% and 99% increases, and those with impaired GT 106% and 128%. Most of the increases in total and free IGF-I appeared within the first 2 weeks of GH administration (73% and 85%, n=6), approaching upper normal limits in all patients. A slight and temporary reduction in insulin sensitivity was caused by a reduction in NOGM. The results from this underpowered pilot study indicated that 0.7 mg/d of GH may be relevant for the treatment of HALS, independent of the patient's weight and glucose metabolic status (Haugaard et al., 2006a).

We later completed a 40-week randomised placebo-controlled study (www.clinicaltrials.gov; NCT 00119769) that included 46 HAART-treated HIV patients, half of whom were diagnosed with HALS. This study indicated an interaction between body weight and the morphological and immunological effects of rhGH; a weight-dependent effect of a fixed dose of 0.7 mg/d rhGH was observed (B. R. Hansen et al., 2009). Despite this interaction, the net treatment effect of rhGH therapy in the HALS group corresponded to a 25% reduction in VAT and a 19% reduction in trunk fat. The beneficial fat redistribution in the GH group occurred without concomitant changes in subcutaneous fat in the abdomen or extremities. Also, IR, GT, and total plasma cholesterol and triglycerides did not significantly change during the intervention; rhGH therapy was well tolerated (B. R. Hansen et al., 2009). These results support another randomised, double blind, placebo-controlled trial of 56 patients with HIV, abdominal fat accumulation, and reduced GH secretion. The patients were randomly assigned to receive either subcutaneous GH or matching placebo titrated to the upper quartile of the normal IGF-1 range. The average dose in the study was 0.33 mg/d. The treatment effect was the differ-

ence in the change between the GH and placebo groups: significantly reduced visceral fat and truncal obesity, triglycerides, and diastolic BP, but 2-h glucose levels in GT testing were increased. Adverse events were not increased (Lo J et al., 2008).

A few other studies have looked at the use of GH secretagogues as GH-releasing hormone analogues to induce a more physiological release of GH and avoid the side effects associated with high-dose GH treatment. A large placebo-controlled study of 412 patients with HIV (86% men) and an accumulation of abdominal fat received a daily subcutaneous injection of either 2 mg tesamorelin, a GH–releasing factor analogue, or placebo for 26 weeks. The primary end point was the percent change in visceral AT from baseline, which decreased by 15% in the tesamorelin group and increased by 5% in the placebo group. The secondary end points were effects on lipid levels. Triglycerides and total cholesterol decreased significantly, and the total cholesterol to HDL-cholesterol ratio also improved significantly in the tesamorelin group. However, these findings vary within some studies with no significance (Koutkia P et al., 2004). Adverse events did not differ significantly between the two study groups, but more patients in the tesamorelin group withdrew from the study because of an adverse event. No significant differences were observed in glycaemic measures (Falutz et al., 2007). In the 26-week extension phase, the long-term safety and effects of tesamorelin were evaluated. Patients originally on tesamorelin were re-randomised to 2 mg tesamorelin or placebo, whereas patients originally on placebo were switched to tesamorelin. Treatment with tesamorelin was generally well tolerated and resulted in sustained decreases in VAT and triglycerides over 52 weeks without aggravating glucose. Though the effects on VAT were sustained during treatment, these effects did not last beyond the duration of treatment (Falutz et al., 2008). A systematic review of 10 RCT´s indicated that GH axis treatments are effective in reducing VAT and increasing LBM in patients with HALS (Sivakumar et al., 2011).

Immunostimulation and growth hormone
Increasing evidence in the literature suggests that HIV-infected patients retain qualitative and quantitative deficits in the immune repertoire despite effective antiretroviral therapy (Connors et al., 1997; Gea-Banacloche et al., 2000). In line with this observation, a number of patients with adequate control of HIV replication have been shown to not reach an optimal immunological response with a substantial gain in CD4+ cells (Teixeira et al., 2001).

A major challenge will be to improve and stabilise immune reconstitution in these patients. A number of studies have investigated immune modulation in HIV-positive patients on HAART. Cytokine therapy is an obvious choice due to the ability of cytokines to increase cell-mediated immunity. IL-2 is responsible for peripheral CD4 T-cell stimulation and differentiation and has been shown to increase the mature CD4+ cell count (Pett and Emery, 2001). However, two large randomised studies of IL-2, ESPRIT (Emery et al., 2002), which enrolled 4,111 people with CD4 counts over 300, and SILCAAT (INSIGHT-ESPRIT Study Group, 2009), which enrolled 1,695 people with CD4 counts between 50 and 299, did not show any clinical benefits compared to cART therapy alone. The presence of IL-2 led to a faster increase of CD4+ immune cells, but these cells were less functional than the CD4+ cells regenerated naturally in HAART patients. In addition, frequent adverse effects associated with IL-2 administration may have counterbalanced any benefit from CD4 increases, indicating a reduced potential for

the clinical application of this drug. These trials have raised concerns over immune-based therapy development in HIV.

Other approaches for modulting or increasing naive, as well as mature, CD4 T cell numbers have been examined (Smaniotto et al., 2011). A number of in vitro studies and animal studies have investigated the immunomodulatory functions of GH and IGF-1 and demonstrated that they play key roles (Clark, 1997; Goff et al., 1987). Few clinical studies have investigated the effect of GH on immune function in HIV-infected patients. A small study of five HIV-infected patients receiving a high dose (3 mg/d) of rhGH for 12 months showed increased thymic mass and increased number of naive CD4+ T-cells in the patients (Napolitano et al., 2002). A later study of 12 HIV-infected patients suggested immunological improvements following rhGH administration (Pires et al., 2004). In another open-label crossover study, 21 HIV-positive patients were treated with 3 mg rhGH/d for 6 months and 1.5 mg rhGH/d for another 6 months; they showed increased thymic density, T cell receptor rearrangement excision circles (TREC) in peripheral blood mononuclear cells, and a number of naive CD4+ and CD8+ cells (Napolitano et al., 2008). However, as in the other studies using supraphysiological GH regimens, adverse events were frequent and 9 of the 21 patients dropped out of the study, compromising the interpretation of the data and hampering the clinical applicability of this dose.

We extended the open-label long-term pilot study. The six male Caucasian HIV positive patients with HALS was treated with 0.7 mg rhGH injected subcutaneously between 13:00 and 15:00 daily over 60 weeks, followed by 80 weeks of 0.4 mg/d rhGH (Paper X: Andersen et al., 2010). Over the 140–week period, IGF-I increased substantially to the upper physiological range. In the first period, a 2–2.5-fold increase in total and free IGF-I to the upper limit of the physiological range was achieved. In the second period, total and free IGF-I decreased, but total IGF-I remained approximately 1.5-fold increased compared to baseline. The increase in IGF-1 may be associated with T-cell restoration in HIV patients on stable HAART. We also observed that the mean CD4 T-cell number increased by approximately 35% during the study, and a significant effect was observed after only 36 weeks of therapy with 0.7 mg/d rhGH. The effect of rhGH on the immune response is probably not mediated solely through IGF-I but, at least in part, by an adequate IGF-I response. In a study in rodents, IGF-I injected alone or in combination with GH resulted in more pronounced growth of the thymus and spleen compared to GH monotherapy (Clark 1997). Also, the CD4 T-cell number in the spleen of the rhesus macaque monkey was reported to increase to higher levels upon combined GH and IGF-I treatment than by GH or IGF-I alone (LeRoith et al. 1996). In the present study, the increase in total and bioactive (free) IGF-I to the upper limit of the normal physiologic range with 0.7 mg/d rhGH treatment suggests that this rhGH dose was optimal. Higher doses of rhGH, which increase IGF-I to supraphysiological levels, are associated with severe side effects, including diabetes, myalgia, arthralgia, and cancer (Chokkalingam et al. 2001; Kotler et al. 2004; Lo et al. 2001; Napolitano et al. 2002). One study showed that a lower dose of rhGH (0.4 mg/d) for 6 months increases IGF-I levels by 45% (D'Amico et al. 2006e). These findings suggest an approximately linear dose-response relationship between the rhGH dose and IGF-I response in HIV patients using rhGH in physiological doses. One limitation of the present pilot study was that we did not measure additional parameters, such as T-cell restoration.

In the 40 weeks randomised, double-blind, placebo-controlled study we aimed to validate the preliminary data presented in 2005 (Hansen et al. 2005.). This study randomised normoglycaemic HIV patients on stable HAART to either 0.7 mg/d rhGH or placebo for 40 weeks, followed by an open-label period of 48 weeks during which all patients received 0.4 mg/d GH. The main end points were the immune response in terms of mature and naive CD4+ and CD8+ T-cells, thymic size and density, and T cell receptor rearrangement excision circles, all of which increased significantly in the GH group, but the increase in mature T cells did not reach significance. This lack of significance was partly caused by an interaction of weight and GH, such that a body weight below the median of 72.5 kg was associated with an enhanced effect of GH on both mature CD4+ and CD8+ cells (B. R. Hansen et al., 2009).

The HIV-1-specific interferon (IFN)-γ-producing T cells were estimated by peptide-based enzyme-linked immunospot (ELISPOT) assays at baseline and week 40. Individuals who received rhGH demonstrated increased responses to HIV-1 Gag overlapping 20mer and Gag 9mer peptide pools at week 40 compared to baseline, whereas patients who received placebo showed no functional changes. Combining the data on CD4+ T cell receptor rearrangement excision circles and on thymic density (B. R. Hansen et al., 2009) . Patients with the most robust responses in the ELISPOT assays had improved thymic function following rhGH administration. T cells from these robust responders were characterized further phenotypically, and showed decreased expression of activation and apoptosis markers at week 40 compared to baseline. Furthermore, CD4 and CD8 T cell populations were found to be shifted towards an effector and central memory phenotype, respectively (Herasimtschuk et al., 2013). We have later estimated the effect of GH on the low-grade inflammation, where we have shown a decrease in suPAR level after 40 weeks of treatment (Lindboe J B et al 2015, submitted).

Later tesamorelin, a GH–releasing hormone analog has been examined in a double-blind, randomized, placebo-controlled trial among 50 cART-treated HIV-infected men and women with abdominal fat accumulation. After 6 months treatment a significant reduction in visceral fat and additionally a modest reductions in liver fat, was observed. Further studies are needed to determine the clinical importance and long-term consequences of this study (Stanley TL et al., 2014).

3.5. LIMITATIONS

Cohorte study
Acknowledging the limited statistical power of our small cohorte study and the multiplicity of end points measured, the data have been confirmed in other studies.

Growth hormone pilot study
The open-label pilot study with six HALS patient described in the present thesis has several limitations, including the absence of a control group.

The injection of rhGH between 1 pm and 3 pm is inconsistent with the normal timing of rhGH injection (at 8pm) for patients with GH (Jorgensen et al. 1990). The evening rhGH injection for GH-deficient patients has been chosen because it induces an increased level of GH during the night and introduces a pulse of GH in the late evening, mimicking the natural diurnal pattern of GH secretion and concentration (Jorgensen et al. 1990). However, HIV patients on HAART are not likely to display overt GH deficiency (Koutkia et al. 2004b). The injection of rhGH when endogenous GH secretion is low was speculated to introduce an additional pulse of GH. This principle could, in theory, enhance the effect of rhGH injection on IGF-I production and the synergistic effect of IGF-I and GH on immune reconstitution. Indeed, these findings showed for the first time that HIV patients on HAART secrete less GH from noon to early evening compared to the rest of the day, proving that the rhGH was injected during a period of low endogenous GH secretion. Determining whether this principle positively impacts immune reconstitution and the rhGH-induced side effects in HIV patients on HAART requires further evaluation.

4. CONCLUSIONS AND PERSPECTIVES

The ongoing discussion about what has to be recognised as the "hen or egg" morphological changes in fat tissue and metabolic disturbances makes it unclear whether HALS has a real consequence. Emerging data suggest that, despite the high plasticity of both the metabolic and immunological pathways within the human body, their regenerative capacities have boundaries, limiting the reversibility when cell exhaustion becomes evident and morphological damage has occurred.

However, 18 years of intense research have not been futile, as pieces of the puzzle are starting to come together. The whole picture of this spectacular syndrome is being unravelled little by little, revealing a multitude of factors (immunological, genetic, viral, and pharmaceutical) that could potentially create a vicious cycle and contribute to the appearance and progression of the metabolic and morphological features of HALS. Indeed, all research points away from generalisation, although some aspects of HALS are clearly related to certain drugs as hypertriglyceridaemia and lactate acidosis, and IR and low-grade inflammation may be two of the main parameters that determine the appearance and outcome of the syndrome.

As is the case with syndromes within other areas of medical history, syndromes will always be a discussion within "lumbers and splitters". Our duty as a part of the health care system is to act upon the single patient and treat their symptoms, not wait for a firm conclusion in the ongoing scientific discussion. The discussions of whether the visceral fat accumulation can be seen upon as "ectopic fat" -a consequence of dysfunctional peripheral fat or the visceral fat hypertrophy is based upon a different mechanism are being examined both in the settings of pathophysiological study methods and mathematical as a latent class model with two or three compartment (Petersen Janne submitted 2015).

With regard to the pathogenesis of HALS the papers and refrences presented in this thesis, have to be seen in a context of the natural history of the HIV-disease due to the change over the last decades of the CAART. From NRTI containing treatment regiments to combinations of drugs, which are less mithochodrie toxic changing the development of the HIV disease and the metabolic and the morphologic complications. From a syndrome consisting of three phenotypes: atrophy, mixed-type and hypertrophy to become merely a syndrome of mixed and hypertrophy (Guaraldi et al., 2014) and as such a model of biological ageing (Pathai

et al., 2014) and fraility (Mohler et al., 2014). HIV may possibly induce an accelerated process of immunosenescence and systemic ageing, "Inflamm-ageing", in which stress response and inflammation are a part of an integrated response, crucial for survival (Ginaldi et al., 2005; Ottaviani and Franceschi, 1998). The inflammatory cytokines and the anti-stress response are up-regulated, inducing immunodeficiency in the absence of HAART and acting as a factor in the development of HALS in the presence of HAART. HIV-associated lipodystrophy syndrome has similarities with "sad buttock syndrome" a prominent feature of ageing (Figure 2).

At least two possible strategies that might help delaying "inflamm-ageing" can be considered: 1) block or minimise inflammatory and oxidative stress responses or 2) restore the regenerative capacity of the immune system. These strategies might be taken into consideration sequentially or together under careful control, as any manipulation of the immune system is a double-edged sword.

This "inflamm-ageing" hypothesis has been indirectly examined in different settings. In a small cohort of HIV patients where the prevalence of lipodystrophy was 32.4%, physical activity was considered an independent protective factor against the onset of HALS (Justina et al., 2014) and in the MACS body composition substudy, findings suggest that inflammation may contribute to declines in functional performance, independent of age (Crawford et al., 2013). In addition, the hypothesis has been examined in trials of GH treatment to reconstitute the production of the thymic microenvironment and stimulate naive CD4+ T-cells. We and other groups report that administration of rhGH in patients receiving effective HAART resulted in a greater improvement of T lymphocyte function than observed with HAART alone, and theseresults also provide further evidence that such an approach could also reduce levels of immune activation.(B. R. Hansen et al., 2009; Herasimtschuk et al., 2013; Napolitano et al., 2002) or by administration of cytokines, such as IL-2, to expand circulating

Figure 2. Photo of sad buttock (with permission)

CD4+ cells (INSIGHT-ESPRIT Study Group, 2009; Kovacs et al., 1996). In mouse models, IL-7 fusion protein has been shown to induce thymopoiesis (Henson et al., 2005).

We have recently performed immunophenotypic analysis of CD8 T-cells and examined differentiation and maturation markers, and to the expression of the inhibitory receptors programmed death-1 (PD-1) and killer cell lectin-like receptor G1 (KLRG1) in 75 subjects: 60 subjects with HIV-infection (HIV+), and 15 age-matched healthy controls. This cross-sectional study indicated, that HIV+ subjects displays a premature ageing of the CD8 T- cell compartment. The altered distribution and senescence of CD8 T- cells reflect signs of ageing including AT distribution and IR. However, PD-1 and KLRG1 expression were not affected by HIV-infection, but associated with age, abdominal visceral AT area and muscle mass (Tavenier J et al. submitted 2015).

In patients with HALS, more randomised controlled trials are needed to establish the role of PI, NRTI, NNRTI immunomodulators, and other agents in the prevention of diabetes and CVD. Importantly, the advances in knowledge about the pathogenesis of type 2 diabetes, adipositas, and CVD might not be valid in relation to patients with HALS. Nevertheless, the knowledge gained from trials in HIV-negative populations has provided a tool for identifying molecular responses and mechanisms in HALS, and may help identify new approaches for its treatment and prevention. Also, the data obtained from in vitro studies provide a platform for further studies on the syndrome. Certainly, knowledge is needed to enable the identification of modifiable risk factors in patients who have not yet had complications such as HALS, diabetes, and coronary heart disease. One way of obtaining such information could be to identify specific serum biomarkers of inflammation and oxidative stress in HALS. Critical to evaluating biomarker performance is the ability to distinguish between measurement system variance and inherent biological variance, because it is within the latter that healthy background variability and high-value, disease-specific information reside. The increasing worldwide prevalence of HALS calls for continued research efforts involving basal, animal-based, and human studies to improve and renew patient care.

"Imagination is more important than knowledge."
 (Albert Einstein)

5. SUMMARY

HIV-associated Lipodystrophy Syndrome frequently presents as a relative lack of peripheral adipose tissue storage combined with an increase in visceral fat, associated with insulin resistance and dyslipidaemia. This thesis discusses explanations for the links between abnormalities in glucose metabolism, the steroid synthesis pathway, the growth hormone-insulin growth factor-1 axis, and chronic changes in adipose tissue distribution. Specifically, the mechanisms by which low-grade inflammation may affect the normal stimulatory effect of insulin on glucose and fat storage are reviewed. We propose that both chronic low-grade inflammation from HIV infection and treatment with HAART trigger cellular homeostatic stress responses with adverse effects on glucose metabolism. The physiological outcome is such that the total energy storage in the adipocytes is decreased, and the remaining adipocytes resist further energy storage. The excess circulating and dietary lipid metabolites, normally metabolised by adipose tissue, are deposited ectopically in the muscle, liver, or visceral adipose

tissue, where they impair insulin action. This deposition of lipid metabolites leads to a vicious circle of insulin resistance and lipotoxicity leading to lipoatrophy or a mixed-type with increased visceral adipose tissue and a clinical phenotype of HIV-associated lipodystrophy syndrome with an elevated waist-to-hip ratio. This HIV-associated inflamm-ageing syndrome can provide a platform for further studies in HIV-infected patients and act as a model for biological accelerated ageing.

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