General Discussion
Introduction
The introduction of highly active antiretroviral therapy (HAART) in 1996 has resulted in an enormous decline in mortality of patients living with HIV-1 in the developed world. [1,2] This initially led to a state of euphoria resulting from the belief that HIV could be turned into a chronic disease, since adequate control of viral replication was within reach. Initially, it even seemed that after 2 to 3 years of HAART HIV-1 could be completely eradicated resulting in a complete cure from HIV-1 infection. [3] This success was also responsible for the fact that the stigma with respect to HIV/AIDS decreased in the general population. [4] However, the last years it has become clear that the different aspects of the lipodystrophy syndrome potentially undermine parts of these enormous successes booked with the introduction of HAART. A recent study demonstrated that lipodystrophy has major psychosocial implications for patients suffering from this condition.[5] Furthermore, several studies have demonstrated that lipodystrophy has impact on treatment adherence and thereby might lead to the emergence of viral drug resistance.[6-8] Even more importantly it seems likely that several aspects of the syndrome might diminish life-expectancy of HIV-1 infected patients, by increasing the risk of cardiovascular disease (CVD).

Hyperlipidemia

In Chapter 3 of this thesis we demonstrate that different antiretroviral drug regimens are associated with changes in lipoprotein profiles that might distinguish them with respect to their associated risk on the incidence of CVD. In this open-label randomised study (Atlantic study), treatment with two nucleoside reverse transcriptase inhibitors (NRTIs), combined with the addition of either a protease inhibitor (PI), a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a third NRTI was compared.[9] Twenty-four weeks of PI-based therapy resulted in modest increases in total-, LDL-, and HDL-cholesterol, resulting in no change in the ratio of total over HDL-cholesterol. This ratio is a strong predictor of
cardiovascular disease in the general population.[10,11] In contrast, in the patients randomised to the NNRTI nevirapine the ratio of total over HDL-cholesterol decreased by 14%, due to a striking 49% increase in HDL-cholesterol and a modest increase in LDL-cholesterol. Recently, these findings were supported by the results from a large international, multicenter cohort study. In contrast to PI-based regimens and analogous to the results of Chapter 3, the patients in this cohort study who initiated first line NNRTI-based therapy had significantly higher concentrations of HDL-cholesterol. [12] In Chapter 4 the theoretical impact of these differential effects on lipoprotein profiles for estimating future cardiovascular risk are discussed, particularly for those patients, who prior to the initiation of antiretroviral therapy, already are at increased risk for developing cardiovascular disease. We propose that it would seem reasonable in the latter group to initiate treatment with a NNRTI-based regimen rather than a PI-based regimen assuming no other convincing arguments exist favouring one over the other.

Within the class of NNRTIs both efavirenz and nevirapine elevate HDL-cholesterol.[13-19] In the recently presented 2NN study, which is a head to head comparison of first line therapy with stavudine and lamivudine together with either nevirapine, efavirenz, or both, it was demonstrated that the regimen containing nevirapine led to a more pronounced rise in HDL-cholesterol when compared to efavirenz (+0.37 mmol/l in the NVP arm compared to +0.24mmol/l in the efavirenz arm, p <0.001).[20] In the nevirapine arm the ratio of total cholesterol over HDL-cholesterol decreased, while this was not the case in the efavirenz arm, possibly as a result of the smaller increase in HDL-cholesterol.

Disentanglement of the mechanism by which these two different NNRTIs elevate HDL-cholesterol may be of interest even for patients outside the field of HIV-infection. A HDL particle consists of one apolipoproteinAI (ApoAI) molecule, that is produced in the liver. ApoAI is loaded with cholesterol from the plasma and peripheral cells and is thereby responsible for the so-called reverse cholesterol transport. This
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cholesterol may then be taken up in the liver, through action of the enzyme cholesterol ester transporting protein (CETP) and subsequently is excreted in the bile. Based on the finding that CETP mass did not change over time in the patients randomised to treatment with nevirapine in Chapter 3, it seems unlikely that the increase in HDL-cholesterol resulted from a decreased clearance of HDL-cholesterol. Since the rise in HDL-cholesterol was associated with a 19% increase in ApoAI concentrations, a more likely explanation therefore seems that the NNRTIs stimulate apoAI production. Although both NNRTIs were engineered to inhibit HIV-1 reverse transcriptase their molecular structure is completely different.[21,22] This suggests that the molecular target for raising HDL cholesterol is likely to resemble the binding site of the NNRTIs on HIV-1 reverse transcriptase. Therefore studies should be performed focussing on the resemblance of this binding site with crucial enzymes involved in HDL cholesterol metabolism. Determining the mechanism by which the NNRTIs increase HDL cholesterol might give an important impulse to the development of drugs designed to raise HDL-cholesterol for the treatment of hyperlipidemia in the general population.

With respect to the mechanisms by which PIs raise total- and LDL cholesterol several studies have been performed. In healthy volunteers treatment with the PI ritonavir led to elevations of triglycerides, apolipoprotein B and VLDL-cholesterol.[23] This suggests that PIs, independent of the presence of HIV-1 infection, directly contribute to the potentially deleterious effects on lipids. Since a substantial part of PI-containing HAART-regimens contains low doses of ritonavir, that are used to increase the plasma drug concentrations of several PIs, a large proportion of PI-treated patients are likely to develop hyperlipidemia. Another study showed that certain genetic ApoC-III polymorphisms are associated with higher PI-induced elevations in triglycerides, demonstrating that genetic predisposition may also play a role in the development of the hyperlipidemia associated with the use of PIs.[24]
The uptake of oxidised LDL into macrophages is crucial for the formation of atheromatous plaques. This uptake is mediated by scavenger receptors such as CD36.[25,26] Dressman et al demonstrated that the different PIs upregulate scavenger receptor CD36 specifically on macrophages.[27] More interestingly, in a transgenic LDL-receptor knockout mouse-model they have shown that low doses of PIs, in the absence of an effect on plasma total- and LDL-cholesterol concentrations, still significantly increased the number of atherosclerotic lesions in the aortas of these animals. Interestingly, another study showed a downregulation of CD36 in exactly the same human macrophage cell line as studied by Dressman[28], indicating the possibility of protection against the atherogenic effects of oxidised LDL. Therefore larger studies are necessary to see whether PIs influence CD36 expression on macrophages in humans, and thereby may promote atherogenesis independently of elevated plasma lipid concentrations.

There are no rational reasons to assume that HIV-1 infected individuals with HAART-induced hyperlipidemia are not at increased risk of developing CVD. A large prospective cohort study concluded that every additional year of use of HAART was associated with a 26% increased risk of CVD.[29] Although this seems a modest increase one has to take into account that this relatively young patient population currently is expected to have to use treatment for the remainder of their life. Thus, particularly in those with pre-existing CVD risk factors a potentially high incidence of CVD may emerge with time. Moreover, several published cross-sectional studies have suggested that PI-based therapy is associated with a decrease in endothelial function and an increase in carotid intima media thickness.[30-33] In contrast, one published retrospective cohort study by Bozzette et al [34] did not demonstrate any increased risk for CVD in HIV infected patients.
Characterisation of metabolic changes in lipodystrophy

In HIV-infected patients with lipodystrophy several other aspects of metabolism, besides lipoprotein metabolism, are disturbed to a great extent. In Chapter 5 and 6 we show that HIV-1 infected patients using PI-based therapy with established lipodystrophy syndrome have increased rates of postabsorptive glucose production and lipolysis. Furthermore, the insulin-stimulated peripheral glucose uptake is decreased, reflecting peripheral insulin resistance, and in addition we have shown that these patients also have hepatic insulin resistance and impaired suppression of lipolysis by insulin. Finally, norepinephrine concentrations are increased suggestive of an increased overall sympathetic tonus.

Although PIs acutely, but reversibly, disturb glucose metabolism by the inhibition of the activity of the glucose transporter GLUT-4, as was elegantly demonstrated in vitro [35,36] and in vivo in healthy volunteers [37,38], in patients with antiretroviral associated lipodystrophy 96 weeks after the withdrawal of PIs no improvement in insulin stimulated peripheral glucose uptake occurs as described in Chapter 8 of this thesis. Visceral fat accumulation and peripheral fat loss in these patients remained unchanged. Apart from the direct effects, which PIs have on glucose metabolism, disturbances in fat distribution are likely to also affect glucose metabolism, as is clearly demonstrated in HIV-1 negative patients suffering from congenital lipoatrophic syndromes. [39] Moreover, visceral fat accumulation is a well-known risk factor for the induction of insulin resistance. [40] Intracellular glucose metabolism however did improve after PI-withdrawal, reflected by an increase in postabsorptive and insulin-stimulated glucose oxidation. Furthermore, the increased rate of lipolysis gradually returned to the levels measured in healthy volunteers, possibly resulting from a decrease in norepinephrine concentrations seen after PI withdrawal, which may reflect a decrease in the overall sympathetic tonus.
In addition to the PIs, the NRTIs also are also undisputedly associated with the development of lipodystrophy, as illustrated by the synergistic effects of the combined use of both PIs and a NRTI on the development of lipodystrophy described in Chapter 2. As a potential mechanism underlying this association "NRTI induced mitochondrial toxicity" resulting in a depletion of mtDNA of adipocytes has been suggested.[41] However, as discussed in Chapter 7, this mechanism seems to be at most partially associated with the development of lipoatrophy. In this study we found a very large difference in the prevalence of lipodystrophy in patients treated with the NRTI stavudine, compared to patients treated with zidovudine (82% vs 9%), without any differences in the mtDNA content of subcutaneous fat biopsies taken from the affected site. This suggests that an alternative mechanism might be involved other than mitochondrial DNA depletion directly at the level of adipocytes.

In contrast to our study, two case control studies did show that mitochondrial DNA content in peripheral fat biopsies was lower in patients with lipoatrophy. [42,43] It is important to note that patients with lipoatrophy in these studies had longer exposure to NRTIs than controls, thereby potentially biasing the conclusions. Nevertheless, the 'mitochondrial toxicity of adipose tissue' hypothesis should not be abandoned as yet, as it may still play a contributory role in the development of lipodystrophy.

In Chapter 6 we show that patients with lipodystrophy while using PIs have increased norepinephrine concentrations compared to untreated HIV-1 infected patients. This seems likely to reflect an increased sympathetic nervous system tonus. Neuroanatomical and physiological evidence for sympathetic innervation of adipose tissue has been published, suggesting a role for this branch of the autonomic nervous system in the regulation of lipolysis.[44,45] Recently, our group demonstrated that intra-abdominal and subcutaneous fat pads are
innervated by separate sympathetic and parasympathetic motor neurons.[46] Furthermore, somatotopy within central parts of the autonomic nervous system with respect to the innervation of different fat depots was demonstrated. Taken together, this strongly suggests that the central nervous system is capable of controlling fat distribution by differentiating sympathetic and parasympathetic output to fat depots. Based on the somatotopy we postulate that a differential effect by antiretroviral agents on the nuclei involved in fat distribution in the central nervous system might lead to a disturbed balance between parasympathetic and sympathetic tonus in favor of the sympathetic nervous system in peripheral fat resulting in peripheral lipoatrophy. Together with a predominant activation of the parasympathetic system in the visceral fat compartment this might result in fat accumulation in that compartment. Alternatively, the increased rate of lipolysis in peripheral fat following an selective increase in the sympathetic tonus solely in this fat compartment might lead to an increased uptake of fat in visceral fat depot, since this ensures energy homeostasis. This provides the first model that is able to explain the selective loss of peripheral fat and simultaneous accumulation of visceral fat as occurs in lipodystrophy. Central to this hypothesis is the influence by the involved agents on select neurons within autonomic nuclei. Additional studies have to be performed to assess the penetration of the different antiretrovirals in the nuclei crucial to the innervation of fat. Within these nuclei functional changes need to be demonstrated that should be coherent in explaining the changes in fat distribution.

**Pathogenesis of the lipodystrophy syndrome**

Based on currently available data we believe that there are several different mechanisms involved in the development of the metabolic aspects of the lipodystrophy syndrome. These different mechanisms have been discussed previously and are summarised below.
1. An acute PI-induced upregulation of CD36 on macrophages
2. An acute PI-induced hyperlipidemia
3. An acute PI-induced GLUT-4 inhibition
4. Altered autonomic control of adipose tissue
5. Mitochondrial toxicity
6. Exaggerated metabolic deterioration resulting from lipoatrophy and/or fat accumulation

To provide an overview of our current insight concerning pathogenesis, the different mechanisms are illustrated in relation to each other in Figure 1. The main message is that no single mechanism is able to explain all abnormalities observed in patients, but that the antiretroviral drugs are likely to induce changes in different biological systems and in sequence.

**Figure 1** Pathogenesis of the antiretroviral therapy (ART)-induced lipodystrophy syndrome (dotted lines illustrate uncertain relationships)
Lipodystrophy syndrome: future directions

Given the multifactorial pathogenesis of the lipodystrophy syndrome a long way lies ahead before these metabolic problems will be overcome. Up until that time a lot of effort needs to be put into proper clinical management of patients suffering from one or more aspects of the syndrome. Reasonable guidance regarding the management of these metabolic complications has recently been provided by an international panel of experts.[47] However, given the disappointing reversibility of the disturbances in glucose metabolism and fat distribution once the syndrome is apparent, more studies should focus on preventive treatment strategies and/or tools that are able to pick up disturbances in fat distribution and/or glucose metabolism at an early stage.

Once the mechanisms underlying the drug-induced metabolic changes are unravelled, new drugs can be engineered with identical potency with respect to the suppression of HIV-1, but with less or no effects on metabolism. Based on currently available antiretroviral drugs several strategic combinations can be considered that might prevent the development of at least some aspects of the lipodystrophy syndrome. For instance, the PI-sparing combination of 2 NRTIs with a NNRTI does not lead to detrimental dyslipidemia and disturbances in glucose metabolism. A good example of this strategy might be the 2 NRTIs lamivudine and tenofovir in combination with the NNRTI efavirenz of nevirapine. Alternatively, regimens without NRTIs may show promise given that such combinations might delay the occurrence of disturbed fat distribution, as we demonstrated in Chapter 2 of this thesis. In this context, combinations consisting of a PI and NNRTI, for example nevirapine or efavirenz in combination with ritonavir/lopinavir, may be considered. The availability in the near future of novel PIs, such as atazanavir, which unlike any of the currently licensed PIs is not associated with elevations in plasma lipid concentrations [48-50], may render combinations of PI and NNRTIs even more promising.
In the following years several new classes of antiretroviral agents under development are expected to become available. These may include integrase and fusion inhibitors as well as chemokine receptor blockers. All these three classes of agents inhibit HIV-1 replication by different mechanisms than currently licensed classes of antiretrovirals. [51] Since a large part of the metabolic toxicity we currently see seems to be class specific, it might very well be that these novel drugs may not disturb metabolism.
References


35. Murata H, Hruz PW, Mueckler M: **Indinavir inhibits the glucose transporter isoform Glut4 at physiologic concentrations.** *AIDS* 2002, **16**:859-863.


