Metabolic complications of antiretroviral therapy

van der Valk, M.

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Summary
The aim of this thesis was to characterise the different aspects of the lipodystrophy syndrome focussing on glucose and lipid metabolism. In Chapter 1 an historical overview is given on the emergence of the lipodystrophy syndrome in the treatment of HIV-1 infected patients. Lipid changes and alterations in glucose metabolism, lipolysis and energy expenditure in the natural course of an HIV-1 infection and during treatment are reviewed. Two published hypotheses explaining the occurrence of lipodystrophy are discussed.

Initially, the lipodystrophy syndrome was thought to be caused solely by protease inhibitors (PIs). However, soon thereafter it became clear that patients who had never been exposed to PIs, but solely to nucleoside reverse transcriptase inhibitors (NRTIs) also developed this syndrome. The exact contribution to the development of lipodystrophy of these two classes of antiretroviral agents remained unclear. The Prometheus study (Chapter 2) provided an opportunity to examine this question. In this multicenter, open-label, randomized study the occurrence of lipodystrophy, as reported by physicians, was determined in patients randomly assigned to treatment with either PIs alone (ritonavir (RTV) and saquinavir (SQV)) or PIs combined with an NRTI (RTV/ SQV and stavudine(d4T)). Lipodystrophy was reported in 29 of 175 (17%) patients during 96 weeks of follow up. Overall, it was reported significantly more frequently in patients randomized to RTV/SQV/d4T (22/88; 25%) than in patients randomized to RTV/SQV alone (7/87; 8%) (P < 0.003). When the analysis was limited to patients without any prior antiretroviral experience, lipodystrophy likewise was significantly more frequent in patients randomized to RTV/SQV/d4T (12/50; 24%) than in those randomized to RTV/SQV (2/44; 5%) (P < 0.008).

Based on this randomized clinical trial we concluded that NRTIs contribute to the development of antiretroviral therapy-associated lipodystrophy. The low incidence of lipodystrophy in patients with no or limited NRTI-exposure supports further evaluation of NRTI-sparing regimens as alternatives to current antiretroviral regimens.
Protease-inhibitor containing antiretroviral therapy for the treatment of HIV-1 infection is associated with elevated triglyceride and LDL-cholesterol levels, which may expose patients to an increased risk of cardiovascular disease (CVD). In Chapter 3 we report the lipoprotein profiles of a representative subset of treatment-naive patients included in the Atlantic Study. This randomised multicenter open-label study compared patients treated with d4T and didanosine plus the addition of either the non-nucleoside reverse transcriptase inhibitor (nNRTI) nevirapine (NVP), the PI indinavir (IDV) or the NRTI lamivudine. We observed a striking increase in HDL-cholesterol (49%), apolipoprotein (apo) AI (19%), lipoprotein (Lp) AI (38%) and HDL particle size (3%) in the nevirapine treated patients (n=34) at week 24. Much less pronounced changes in these parameters were seen to a similar extent both in patients receiving lamivudine (n=39) and IDV (n=41). LDL-cholesterol also increased significantly both in the NVP and IDV arms, but only in the NVP arm was this offset by a significant reduction (14%) in the ratio of total over HDL-cholesterol. Using a multivariate linear regression model, adjusting for CD4+ cell count and plasma HIV-1-RNA both at baseline and during treatment, randomisation to the NVP-containing arm remained significant in explaining the observed changes in HDL-cholesterol and other HDL-related parameters.

Based on this randomized clinical trial we concluded that in HIV-1 infected patients treated with a regimen of stavudine, didanosine and nevirapine changes in lipids and lipoproteins occur that are associated with a sharp decrease in risk for CVD in other settings.

In Chapter 4 the results observed in Chapter 3 are put into perspective. Based on this we illustrate how such differences in the lipoprotein profile, together with knowledge of the presence of other classic CVD risk factors, opens the door for individualised treatment, based on criteria in addition to HIV-1 viral load and CD4-cell count. It is becoming increasingly important to make an adequate CVD risk assessment in each patient both prior to and approximately annually after the initiation of highly active antiretroviral therapy (HAART). In
patients with an already considerable risk of CVD based on traditional risk factors, in particular when it is expected to be difficult to modify these risk factors, starting with either a triple NRTI or an nNRTI-based regimen may be the preferred option given the propensity of such regimens to have either no or potentially even beneficial effects on the lipoprotein profile.

Glucose metabolism in HIV-1 infected patients with lipodystrophy is characterized in detail in Chapter 5. We assessed glucose disposal and its pathways, glucose production, plasma free fatty acid levels, and the degree to which these parameters could be suppressed by insulin. Lipodystrophy was defined as an evident loss of peripheral fat with or without visceral fat accumulation while using PI-based therapy. These results were compared to those obtained in age, gender and body mass index-matched healthy volunteers. Six HIV-1 infected men on PI-based HAART with lipodystrophy were studied. The results were compared to those obtained in 6 matched healthy male volunteers. Insulin sensitivity was quantified by hyperinsulinemic euglycemic clamp. Glucose production and uptake were assessed by tracer dilution employing [6,6-²H₂]-glucose. Under fasting conditions, HIV-1 infected individuals suffering from the lipodystrophy syndrome had a 47% higher glucose production than healthy controls (p=0.025). During insulin infusion (plasma insulin concentrations ~200pmol/l) glucose production was suppressed by only 53% in the lipodystrophy group, and by 85% in controls (p=0.004). Peripheral glucose disposal increased in both groups, but by only 27 % the lipodystrophy group versus 201 % in controls (p=0.004). Consequently, insulin-stimulated total glucose disposal was lower in the lipodystrophy group (p=0.006). Non-oxidative glucose disposal as percentage of total disposal did not differ significantly between groups (63 % in the lipodystrophy patients and 62% in controls). Baseline plasma FFA concentrations were higher (0.60 vs. 0.35 mmol/l; p=0.024), whereas FFA decline during hyperinsulinemia was less (65 vs. 85 %; p= 0.01) in the lipodystrophy group versus controls. Based on these results we concluded that post-
absorptive glucose production is increased in HIV-1-infected patients with lipodystrophy. Moreover, both the ability of insulin to suppress endogenous glucose production and lipolysis, and to stimulate peripheral glucose uptake and its metabolic pathways is reduced, indicating severe resistance concerning multiple effects of insulin.

**Chapter 6** explores the effect HIV-1-associated lipodystrophy has on the rate of lipolysis and on resting energy expenditure, and compares this to data obtained in asymptomatic HIV-1 infected patients, not receiving combination antiretroviral therapy. Furthermore, we compared the lipolytic response to an epinephrine infusion in these two groups. The rate of lipolysis was measured using [²H₃]-glycerol infusion, the resting energy expenditure (REE), by indirect calorimetry, and the response of both to a physiological infusion of epinephrine (~ 15 ng/kg/min) was assessed. Results in 8 lipodystrophic patients were compared to those obtained in 5 age,gender and BMI-matched HIV-1 infected patients (HIV). Fasting glycerol turnover did not differ between the two groups, nor was there a difference in the lipolytic response to epinephrine, although the response in the lipodystrophy group group was delayed (p < 0.001). In the lipodystrophy group fasting REE adjusted for lean body mass was lower and remained lower during epinephrine infusion. Fasting norepinephrine concentrations were higher and remained elevated during epinephrine infusion in the lipodystrophy group. From this study we concluded that the lipolytic response to epinephrine in the LD group was normal albeit delayed. Norepinephrine concentrations were increased in patients with lipodystrophy, indicating increased sympathetic activity. Postabsorptive REE was lower in the patients with lipodystrophy. Together this suggests that antiretroviral therapy associated lipodystrophy normalises the REE but has only minor effects on lipolysis, as a result of concomitant sympathetic stimulation of adipose tissue.
Chapter 7 explored the hypothesis that lipodystrophy syndrome is linked to an antiretroviral agent-induced effect on mitochondrial DNA, thereby impairing mitochondrial function of adipocytes, resulting in apoptosis of these cells, as was previously proposed by Brinkman et al. To study this question we cross-sectionally assessed lipodystrophy by standardized questionnaire, CT- and DEXA scan in patients who, 4 years prior to the current cross-sectional study, had participated in a randomized open-label comparative trial of stavudine or zidovudine-based antiretroviral therapy. MtDNA content was measured in peripheral blood mononuclear cells (PBMC), and subcutaneous adipose tissue taken from the thigh and back. All assessments were performed while unaware of past and current medical history. 28 of the 45 patients who had originally started randomized treatment were included in the current study. Despite comparable exposure to either stavudine or zidovudine (51 and 50 months, respectively) the prevalence of lipoatrophy was significantly greater in patients allocated to stavudine compared to zidovudine (82% versus 9%, p = 0.0001). Likewise, in those allocated to stavudine peripheral fat content by radiographic measures was significantly lower. Although mtDNA content of PBMC decreased after the start of treatment in both groups, (73% and 63% in the stavudine and zidovudine arm, respectively, p=0.01), there was no statistically significant difference in the present mtDNA content of their fat biopsies. Based on these results we concluded that stavudine-containing regimens are associated with a greater risk of lipoatrophy than zidovudine-containing regimen. Both treatments resulted in similarly impressive declines in mtDNA content of PBMC. Nevertheless no differences in mtDNA content of fat were observed. Together, these findings suggest that the differential risk of lipoatrophy observed with different NRTIs cannot solely be explained by differences in mitochondrial DNA depletion directly at the level of peripheral adipose tissue.
In **Chapter 8** the effect on glucose metabolism, lipolysis and fat distribution of 96 weeks of PI-replacement by the NRTI abacavir in eight severe lipodystrophic individuals is described. During fasting and during a hyperinsulinemic euglycemic clamp glucose metabolism and lipolysis were assessed by tracer dilution method employing [6,6-2H2] glucose and [2H5] glycerol, respectively. PI replacement by abacavir in severe lipodystrophic HIV-1 infected patients resulted in a marked reduction of lipolysis and significant improvement of glucose oxidation. In contrast, fasting glucose production improved modestly, while insulin stimulated glucose disposal and fat distribution did not change. Taken together, this suggests that mechanisms in addition to inhibition of GLUT-4 activity are responsible for the changes in glucose metabolism seen in HIV-infected patients with established lipodystrophy.

In the general discussion in **Chapter 9** recent findings with respect to HAART-induced hyperlipidemia and their clinical implications are discussed. In addition, the different aspects of the lipodystrophy syndrome are characterised and potential mechanisms explaining the various aspects of the syndrome are discussed. An alternative mechanism explaining the occurrence of fat redistribution is proposed. Finally, a figure is presented that provides a schematic overview of the different mechanisms involved in the occurrence of the syndrome and their relation with the initiation of HAART.