

2<sup>ND</sup> EUROPEAN SYMPOSIUM

# Clinical Significance of Lipoprotein Disorders in HIV-positive Patients

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## Effect of HIV and its treatment on lipids

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Early in the natural course of HIV infection, as seen in other infections and chronic inflammatory conditions, plasma total cholesterol, HDL- and LDL-cholesterol concentrations decrease. In addition, the size of LDL particles is also reduced resulting in an increase of more atherogenic small dense LDL particles. Finally, when patients progress to AIDS plasma triglyceride concentrations tend to rise. Thus, although not proven, untreated HIV infection in and of itself, may be associated with increased atherogenesis, the risk of which theoretically could be reduced by treating the infection.

Combination antiretroviral therapy (cART) for HIV is often associated with several metabolic abnormalities, including changes in blood lipids. First generation protease inhibitor (PI)-containing regimens were amongst the first recognized as being associated with rises in total and LDL-cholesterol (LDL-C), as well as triglycerides. PI's differ with respect to their effect on lipids, with full-dose ritonavir having the most pronounced effects, most notably on triglycerides. Although still present, ritonavir's effect on lipids is clearly less when used in low dose in order to boost plasma exposure of other PI's, which basically is the sole way the drug is currently still used.

It is key to realize that these changes in lipids observed with PI-based therapy are greater than may be explained by the "restoration of health" phenomenon one would expect to result from suppressing HIV. In view of these effects, and the finding that a high proportion of HIV-infected patients have other risk factors for heart disease, including hypertension, smoking, diabetes, age older than 50 years, male sex, and pre-existing dyslipidemia, it is not surprising that after the widespread introduction of protease inhibitors (PIs) in 1996, the incidence of myocardial infarction (MI) in HIV-infected patients exposed to combination antiretroviral therapy or cART (often including PI) appears to have increased. Although it has now been clearly documented that the risk of MI continues to increase with longer exposure to cART, the absolute risk of MI remains low and does not outweigh the marked effectiveness of combination antiretroviral therapy in preventing HIV-related complications. Thus, clinicians should never withhold cART from any patient with an indication to commence treatment for preventing HIV disease progression.

Interestingly, initiating treatment with non-nucleoside reverse transcriptase inhibitor-containing regimens has been shown to result in a different lipid profile, characterized most notably by marked rises in HDL-cholesterol against the background of lesser rises in total cholesterol, LDL-cholesterol, and triglycerides. The HDL-c increase has been investigated extensively for nevirapine (NVP), and somewhat less for efavirenz (EFV). In both instances the rise in HDL-c results from an increase in the concentration of large HDL particles, which may be expected to provide protection against atherogenesis. Preliminary studies using intima-media (IMT) thickness of the carotid artery as a surrogate marker of atherosclerosis, seem to suggest a lower atherogenic propensity in patients treated with ART containing nevirapine rather than a PI. Currently, there are no robust cardiovascular endpoint data concerning potential differences between different cART regimens with respect to CVD risk.

Clinicians should approach cardiovascular disease prevention and management in a manner similar to that for non-HIV-infected individuals. The evaluation for dyslipidemia and cardiac risk

should be part of the initial patient assessment prior to initiating antiretroviral therapy. Determination of lipid levels, an assessment of cardiovascular risk factors (e. g. smoking, diet, family history), and identification of comorbid and/or preexisting conditions (e. g. diabetes, hypertension, obesity) are integral to this evaluation. In those found to be at significant risk (e. g. according to Framingham or PROCAM algorithms), smoking cessation should be emphasized as an important intervention to improve long-term outcomes. In addition, low-dose aspirin may be recommended to patients who do not have contraindications. Patients should be assisted so that they can follow National Cholesterol Education Program (NCEP) or other guidelines with regard to dietary and lifestyle changes. When diet and lifestyle changes do not produce sufficient improvements in the lipid profile, the use of lipid-lowering agents or changing the antiretroviral regimen should be considered. Preliminary data indicate that substituting atazanavir, abacavir, nevirapine, or efavirenz for a PI and substituting abacavir or tenofovir for stavudine can improve lipid profiles in patients with established dyslipidemia; however, there is also evidence to suggest that changing therapy alone may not achieve goals as set by NCEP or other guidelines. Recent findings from a randomized trial in which subjects with viral suppression to < 50 copies/ml on a PI regimen who also had mixed hyperlipidemia have suggested that adding lipid-lowering therapy—with either pravastatin or bezafibrate—may result in a greater reduction in total cholesterol and triglyceride than switching the PI to a nonnucleoside reverse transcriptase inhibitor (NNRTI)—either nevirapine or efavirenz. The following cautions however apply when considering to add lipid-lowering therapy to antiretroviral treatment which includes a PI. Fibrates, fluvastatin, and pravastatin have low potential for untoward interactions with PIs, but lovastatin and simvastatin should be avoided. Statin-fibrate combinations or atorvastatin should be used with caution. Clinical utility of long-acting niacin may be limited because of its association with worsening insulin resistance.

Finally, if possible within the framework of the primary goal of achieving sustained HIV suppression, the initial choice of antiretroviral regimen should be one that minimizes the risk of treatment-related dyslipidemia, insulin resistance and changing in body fat distribution, particularly in those patients with a high a-prior risk of cardiovascular disease.

### Changes in carotid intima-media thickness in HIV-infected patients included in the ANRS CO3 Aquitaine Cohort, 1999-2004

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Measurement of carotid artery intima-media thickness (IMT) with high-resolution B-mode ultrasound is a non-invasive method for assessing atherosclerosis and tracking its progression. Its fair reproducibility and validity have been demonstrated (Grobbee 1994, Kanters 1997). In the general population, IMT increases with age of about 0.01mm/year (Howard 1993) and an

IMT increase of 0.1 mm increases the risk of coronary events by 11% (Salonen 1993).

In 1999, we designed a prospective study involving participants of the Aquitaine Cohort (Southwestern, France), all infected with HIV-1. In- or out-patients of four participating clinical units were consecutively enrolled if they agreed and signed a consent form. All participants were studied for IMT measurement, HIV risk factors and other well known cardiovascular risk factors on three occasions: a baseline assessment (M0), after one-year (M12) and three years (M36) follow-up. Results have already been reported (Mercié 2002, in press, Thiébaud 2005).

At baseline, 423 HIV-infected patients were enrolled in the study. Median carotid IMT was 0.54 mm [0.50-0.60]. In univariate linear regression, IMT was significantly increased ( $p < 0.05$ ) with older age, male gender, higher body mass index, higher waist-to-hip ratio, increased systolic blood pressure, total cholesterol, glucose disorders and homocysteine, regular smoking and alcohol consumption, lipodystrophy and HAART exposure. In a multivariate analysis, the effect of lipodystrophy and HAART disappeared after adjustment for other cardiovascular risk factors.

At M12, 346 patients were investigated and we observed a significant increase of IMT (+ 0.02 mm,  $p < 0.0001$ ). Surprisingly, only CD4+ cell count at M0 was statistically associated to the variation of IMT from M0 to M12 ( $p < 0.001$ ). We studied the change in IMT throughout follow-up on the 233 patients who had three available measurements over the three-year follow-up period. Between M12 and M36, we observed a significant decrease of median IMT from 0.57 to 0.53 mm ( $p < 0.0001$ ). This decrease of IMT was concomitant with an increased prevalence of treatment with lipid lowering agents and protease inhibitor-free HAART regimens while the smoking prevalence decreased. Only a marginal association between IMT decrease and tobacco smoke was found.

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## What is proved for low HDL as cardiovascular risk factor?

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Numerous clinical and epidemiological studies have demonstrated the inverse and independent association between HDL-cholesterol (HDL-C) and the risk of fatal and non-fatal coronary heart disease (CHD) events. From data of population studies, it has been calculated that every 0.026 mmol/l (every 1 mg/dl) increase in HDL-C lowers coronary risk by 1%. In patients with angiographically assessed CHD this association may even be stronger since the prospective and multicentric European Concerted Action on Thrombosis and Disabilities (ECAT) study, as well as the Baltimore Longitudinal Heart Study identified low HDL-C as the most important biochemical risk factor for coronary events.

However, it is not clear whether this rule can be extrapolated to the whole range of HDL-C. Whereas a low HDL-C level (e. g.  $< 20^{\text{th}}$  percentile) has been consistently found to increase cardiovascular risk, it has not been consistently shown that a high HDL-C level is protective. In certain metabolic conditions a high level of HDL-C is rather associated with excess cardiovascular risk. Hypertriglyceridemic participants of the Copenhagen City Heart Study and the PROCAM Study with high levels of HDL-C were at higher coronary risk than hypertriglyceridemic probands with intermediate HDL levels. Interestingly, although low HDL-C is also significantly associated with reduced life expectancy, HDL-C levels in the fifth quintile were also associated with excess mortality as compared to intermediate HDL levels in the PROCAM and also in a Belgian study population.

Low HDL-C is frequently confounded with hypertriglyceridemia, the presence of small dense LDL, impaired glucose tolerance or overt diabetes mellitus type 2, hypertension, and overweight. Actually, in many populations a low HDL-C is a typical component of the metabolic syndrome or insulin resistance syndrome which precedes the manifestation of the other components including diabetes. Thus, although the association of HDL-C with CHD is statistically independent of other risk factors, a low HDL-C is frequently not the sole risk factor in a given individual. Therefore, elimination of additional risk factors is of equal or greater importance than raising of HDL-C in an effort to reduce overall risk of the individual. This also explains, why in some of the statin trials baseline levels of HDL were strongly associated with the extent of benefit from LDL-C lowering treatment.

Outcomes of several prospective intervention studies with statins and fibrates have been interpreted as proofs for the beneficial effect of increasing HDL-C on CHD prevention. However, it is important to emphasize that these studies used fibrates and statins which exert a broad spectrum of metabolic effects, only one of which is the moderate increase in HDL-C. These studies do only show, that patients with low HDL-C and additional risk factors benefit from treatment with fibrates or statins.

Currently, only half of the coronary events are prevented by the conventional interventions including lipid- and blood pressure lowering therapies. Therefore, effective modification of HDL metabolism remains an attractive target for the development of new regimens of anti-atherogenic drug therapy.

## Changes of HDL concentration, composition and metabolism in HIV-infected patients

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The metabolism of lipids is mediated by particles formed by cholesterol, triglycerides, phospholipids and apolipoproteins. High Density Lipoprotein (HDL) is related to the reverse cholesterol transport, but further pleiotropic effects have been ascribed. Among these, it is noteworthy to describe the strong inverse correlation between the HDL concentration and the occurrence of coronary heart disease, representing an excellent candidate to be studied in HIV-infected patients, since some of these patients present with metabolic disturbances prone to develop atherosclerosis. The so-called HDL concentration refers to the cholesterol content in plasma HDL, but HDL particles are not homogeneous. In fact, HDL could be classified according to size, density, electrophoretic movement and composition indicating different features of each HDL subfraction.

The major protein compound of HDL is the apolipoprotein Apo A-I, but several other functional molecules have also been identified, such as Apo A-II, Apo A-IV, Apo Cs, Apo E, paraoxonase 1 (PON-1), SAA and PAF-AH, that may be related to the beneficial pleiotropic effects ascribed to HDL.

The cornerstone of the HDL metabolism is the liver. The liver secretes Apo A-I and phospholipids to become discoidal HDL. This particle is involved in the recruitment and efflux of cholesterol and phospholipids from cell membranes of peripheral tissues and this process is mediated by the membrane transporter ABCA1. Free cholesterol is then esterified by LCAT and the HDL particle appears spherical (HDL3) and becomes larger (HDL2) as it accepts more free cholesterol. The HDL2-cholesterol is transferred to Apo B containing lipoproteins or being cleared by the SR-B1 receptor mainly located in the liver.

It is especially relevant to consider this phenomena in HIV-infected patients because they are under the influence of a chronic infection plus, most of them, under highly active antiretroviral therapy (HAART), and both conditions are known to influence the plasma concentration of lipids and lipoproteins. HIV-infected patients naïve for HAART presented with low HDL-cholesterol and Apo A-I plasma concentrations. Although data in this issue is scarce, HDL-cholesterol concentration show a trend to decrease as the infection progresses. Further, HDL-cholesterol is positively correlated with the course of CD4 cell counts and conversely, an inverse correlation between Apo A-I and HIV-viral load has been observed.

The HIV-infection and the generalization of HAART have introduced metabolic disturbances, such as lipodystrophy and low HDL-cholesterol concentrations. The use of non-nucleoside reverse transcriptase inhibitors (NNRTI) and recent protease Inhibitors (PI) have been associated with an interesting increase in the HDL-cholesterol concentration. However, our data supports that these changes may be under the influence of the genetic background, and several polymorphisms have been reported to influence the course of the lipid profile, such as MDR-1 C3435T, Apo C-III-455C/T and -482C/T.

The composition, size or distribution of HDL in HIV-infected patients remain poorly studied. In 49 HIV-infected patients, the electrophoretic pattern was similar to that observed in patients with coronary heart disease, consisting with low levels of cholesterol-rich-alpha-1 HDL. After the implementation of a PI regimen, the subfraction pre-beta-1 and pre-alpha-1 of HDL showed a significant increase. These results showed that alt-

hough an increase in HDL-cholesterol concentration was obtained, it does not necessarily mean a better prognosis in the prevention of cardiovascular disease. Similarly, in 20 HIV-infected patients treated with NNRTI, a significant increase of HDL2 using NMR spectroscopy was observed.

PON-1 has been associated with the occurrence of coronary heart disease, probably by inhibiting the LDL oxidation and MCP-1 (a beta chemokine highly related with the atherosclerotic plaque) secretion by the endothelial cells. Interestingly, HIV-infected patients presented with lower PON-1 serum activity than healthy controls, that in turn may confer a higher cardiovascular risk in these patients.

There are some data suggesting the influence of HDL and Apo A-I on the HIV replication and on the course of AIDS disease. We observed in treated patients, that the higher HDL-cholesterol the longer the time with undetectable HIV viral load.

In conclusion, HIV-infection and the usual HAART-schemes influence the modification not only of the cholesterol concentration, but also the distribution of several lipoproteins. This phenomenon may be related to different degrees of risk to suffer from cardiovascular disease, and so a thorough approximation to this metabolic derangements should be implemented. Similarly, more studies should be designed to reach a better understanding of the mechanisms that link HDL and Apo A-I with HIV replication and the course of the acquired human immunodeficiency syndrome.

### Lipoprotein pattern in HIV infected patients before starting antiretroviral therapy with lopinavir/ritonavir (LPV/r)

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#### *Background:*

Protease inhibitor (PI) associated dyslipidemia has raised concerns about increased cardiovascular risk in HIV infected patients.

#### *Objective:*

To get information on changes of lipoprotein pattern (including apolipoproteins) in HIV-infected patients on a LPV/r-containing regime. Now baseline data is available for 65 patients of the Swiss HIV Cohort, previously naïve for PI and NNRTI. Only twelve percent of patients were NRTI experienced (mean exposure: 38.25 months) before LPV/r.

#### *Design and methods:*

Fasting lipid parameters (total cholesterol (TC); triglycerides (TG); HDL-C; LDL-C; VLDL-C; VLDL-TG; VLDL Apo B-

100; LDL Apo B-100; Apo A-I; Apo B; Apo B-48; Apo C-II; Apo C-III; Lipoprotein (a); small, dense LDL; HDL-prec.; HDL3-C; HDL2-C), glucose and uric acid were determined. CD4+ count, HIV-1-RNA, BMI, presence of lipodystrophy, and LPV plasma concentration were also assessed.

#### Results:

The patients of the cohort had a median age of 41 (range 26 - 70) years, were predominantly males (83 %) and had a median BMI of 22.3 kg/m<sup>2</sup> (range 14.4 - 29.7). Median CD4+ count was 173 cells/mm<sup>3</sup> (range 2 - 927), while 74 % had a nadir value of < 200 cells/mm<sup>3</sup>. Median HIV-1-RNA was 5.20 log<sub>10</sub> copies/ml (range 2.19 - 7.83). Lipid parameters (mg/dl) at baseline (mean values ± SD) were:

Total cholesterol	100.4 ± 23.2
Triglycerides	130.4 ± 75.4 (Median 113.5)
HDL-cholesterol (direct)	24.4 ± 6.5
LDL-cholesterol	54.1 ± 17.0
VLDL-cholesterol	21.8 ± 9.0
VLDL-triglycerides	71.5 ± 52.8
VLDL-apolipoprotein B-100	11.8 ± 4.7
LDL-apolipoprotein B-100	33.0 ± 9.1
Apolipoprotein B	44.8 ± 11.8
Apolipoprotein A-I	79.7 ± 16.4
Apolipoprotein C-II	2.3 ± 0.6
Apolipoprotein C-III	5.4 ± 2.3
Lipoprotein (a) – Median (interquartile range)	4.6 (0.1 – 21.00)
HDL-cholesterol (precipitated)	19.0 ± 6.7
HDL3-cholesterol	17.3 ± 6.3
HDL2-cholesterol	1.7 ± 2.7

In the lipoprotein fraction with a density < 1.006 g/ml no ApoB48 was detected. Small, dense LDL (apolipoprotein B-100 in the lipoprotein fraction with a density > 1.44 g/ml) were below detection limit (except two patients). Mean glucose and uric acid were 70.97 and 3.69 mg/dl, respectively.

#### Conclusion:

In this untreated cohort very low concentrations of total cholesterol, LDL-cholesterol, HDL-cholesterol, HDL3-cholesterol, and HDL2-cholesterol were found (as reported in literature). Lipoprotein (a) was substantially lower (median by 65 %) than in treated HIV-positive patients or in a normal population. 29 % of patients had hypertriglyceridemia, no association with small, dense LDL was found. Yet, analysis of composition of VLDL showed prevalence of triglyceride-poor particles (high cardiovascular risk) at triglyceride concentrations between 150 and 220 mg/dl while at higher triglycerides particles were triglyceride-rich. Apo C-II is a stimulator of lipoprotein lipase, while Apo C-III is an inhibitor. Concentration of both Apo C's was low, but the ratio of Apo C-III/Apo C-II was significantly associated with triglyceride concentration. It has to be established in the follow-up how a virologically successful cART including LPV/rtv will influence the profile of lipids, lipoproteins, and apolipoproteins found in untreated HIV infected patients.

## Remnant lipoproteins and atherosclerosis

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Patients at increased risk of coronary artery disease frequently exhibit an atherogenic lipoprotein phenotype characterized by elevated plasma levels of both triglyceride-rich lipoproteins (TRL) and small, dense LDL and low concentrations of HDL-cholesterol. The atherogenic lipoprotein profile is strongly linked to obesity, metabolic syndrome, insulin resistance, familial combined hyperlipidemia, and abnormalities in postprandial lipoprotein metabolism.

Epidemiological data have revealed that plasma TG concentration is an important independent risk factor. A more accurate estimation of the presence of circulating remnant particles was obtained by the analyses of plasma remnant-like particle cholesterol (RLP-C). There is now considerable evidence to indicate that elevated levels of plasma TRL remnants are associated with increased risk of premature CAD. This association may reflect a direct effect of remnant lipoproteins on arterial lipid accumulation and lesion formation or alternatively may represent a characteristic feature of the atherogenic lipoprotein profile. Evidence has been obtained from a number of clinical studies emphasizing the clinical relevance of remnant analysis.

### VLDL concentration, composition and metabolism of triglyceride-rich lipoproteins (remnants) in HIV-infected patients

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HIV-infection itself has a well known effect on serum lipids. With the progression of the immunodeficiency LDL- and HDL-cholesterol levels decrease and triglyceride levels increase. This may be at least partially the effect of increased endogenous interferon levels. The initiation of effective antiretroviral therapy reverses to a certain extent the HIV induced dyslipidemia by increasing LDL-cholesterol and to a lesser extent HDL-cholesterol. However specific effects on lipids can be identified for some antiretrovirals. An increase in triglycerides and VLDL-cholesterol is consistently reported for efavirenz and in particular ritonavir. Ritonavir full dose is rarely used as part of an antiretroviral therapy anymore, but as pharmacokinetic booster ritonavir is involved in daily doses ranging from 100 – 400 mg in virtually all HIV-protease inhibitor regimen except nelfinavir. It has been shown previously for saquinavir and as more recent examples for atazanavir and fos-amprenavir that the co-administration with ritonavir increases triglyceride and VLDL-cholesterol levels compared to the unboosted use of these agents. However the antiviral efficacy of the boosted administration was shown to be superior in all cases and therefore approved as standard therapy. Lopinavir and tipranavir can only be administered in co-administration with ritonavir and may lead to a profound increase in triglycerides in a minority of treated patients.

So far only a few anecdotal reports suggest an association between marked hypertriglyceridemia and pancreatitis or cerebral ischemia qualifying this as rare events given the frequent use of boosted protease inhibitors. A more long term adverse effect may be an increase in cardiovascular events. In general hyper-

triglyceridemia without additional risk factors, e. g. features of the metabolic syndrome, is considered to have a low cardiovascular risk. Recent studies have shown that in hypertriglyceridemia induced by HIV-protease inhibitors VLDL particles have a large size. This phenomenon is found in familial hypertriglyceridemia which is associated with a low cardiovascular risk. However in HIV-associated dyslipidemia a proportion of patients may mimic metabolic syndrome with central adiposity, insulin resistance and low HDL-cholesterol whereas other patients present with hypertriglyceridemia only. In addition the pharmacologic effects of the antiretrovirals do interact with pre-existing conditions such as genetic or dietary induced lipid disorders. In the DAD-study an early increase in myocardial infarction was reported for patients on antiretroviral therapy. One of the multiple baseline risk factors identified was hypertriglyceridemia. The atherogenic effect of hypertriglyceridemia is thought to be mediated by VLDL-cholesterol. However no detailed data on the VLDL-particles are available in this study. In addition because of the limited sample size of cases with myocardial infarction no definite conclusion can be drawn, if hypertriglyceridemia was mainly a treatment effect with antiretrovirals or independent from therapy.

### Genetic Factors and susceptibility to antiretroviral therapy-associated lipid disorders

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Dyslipidemia and lipodystrophy occur in some but not all HIV-infected patients, despite similar exposure to highly active antiretroviral therapy (HAART) and comparable demographic, immunologic, and virologic characteristics. This discrepancy might be related to host genetic factors.

Fauvel (AIDS 2001) showed an association of hypertriglyceridemia with variant alleles of APO C-III (the single nucleotide polymorphisms -482 C>T, -455 C>T, and +3238 C>G) in 60 PI-treated men. Maher (AIDS 2002) and Nolan (AIDS 2003) identified associations of TNF-238 G>A with lipodystrophy, while studies of the contribution of mitochondrial DNA mutations did not show clear-cut results (Martin Am J Hum Genet 2003, McComsey J AIDS 2005).

We have recently reported on the association of APO E and APO C-III variants with hyperlipidemia in a longitudinal study of 329 HIV-infected patients enrolled in the Genetics Project of the Swiss HIV Cohort Study (SHCS) (Tarr J Infect Dis 2005). Carriers of variants of both APO C-III and APO E were at risk for extreme and sustained hypertriglyceridemia when exposed to RTV. The contribution of the APO E  $\epsilon 4$  allele was quantitatively similar to the non-HDL-cholesterol-increasing effect of PI treatment. No association between TNF-238 G>A and lipodystrophy was observed. Our study highlights the issues of appropriate methodology and statistical power in genetic association studies. The influence of the genetic variants on lipids was analysed over >3 years of follow-up, during which patients served as their own controls, through multiple changes of HAART. Longitudinal modeling of lipid levels in large numbers of patients may represent the most powerful approach to quantitating the contributions of genotype and HAART to HIV-related lipid disorders. In contrast, cross-sectional analyses of lipid levels at a single time point during HAART exposure seem to be underpowered to detect the genetic effects.

Longitudinal studies, which include patients enrolled in the SHCS, are underway in our laboratory to evaluate the contribution of 1) variant alleles of additional genes involved in lipid metabolism to HAART-related dyslipidemia, and 2) sequence alterations of mtDNA to lipodystrophy. If pharmacogenetic associations are identified and confirmed in different cohorts of patients, it may soon be reasonable to evaluate the usefulness of genetic analysis to predict which patients will develop lipid disorders when exposed to HAART.

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### What is proved for lipoprotein (a) as cardiovascular risk factor?

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Lipoprotein (a) is composed of low density lipoprotein (LDL) particles additionally containing apolipoprotein (a), a glycoprotein, covalently linked to apolipoprotein B. Plasma concentrations of lipoprotein (a) vary over a 1000-fold-range, and the distribution of levels is highly skewed, with most whites and Asians having low levels. The distribution of plasma levels in blacks is less skewed, and the median level is 2 to 4 times greater than in whites.

A meta-analysis of 27 prospective studies, which included 5436 coronary heart disease cases, demonstrated a consistent, positive association between high plasma levels of lipoprotein (a) and coronary atherosclerosis in whites. Comparison of individuals in the top third of lipoprotein (a) measurements with those in the bottom third in each study yielded a combined risk ratio of 1.6 (95 % confidence interval 1.4 - 1.8). It was concluded that this clear association between lipoprotein (a) and coronary heart disease needs further studies to determine the extent to which this is causal.

A very recent study emphasizes that high lipoprotein (a) predicts risk of angina, and that the risk is substantially increased with high concomitant LDL-cholesterol. In this case control

study 195 men who subsequently developed angina were compared to 195 men who remained free of cardiovascular disease for 5 years. In this study, a lipoprotein (a) concentration measured by ELISA above the 95th percentile ( $\sim 70$  mg/dl) was associated with an almost fourfold risk. Using a commercially available test a strong, a nearly 12-fold increase in relative risk for angina was found at lipoprotein (a) concentrations above 30 mg/dl with concomitantly elevated LDL-cholesterol above 160 mg/dl. Other studies have reported comparable findings, the increase in risk was  $\sim$  fourfold in patients with elevated lipoprotein (a) and concomitantly elevated LDL-cholesterol.

As no lifestyle or drug therapy of elevated lipoprotein (a) exists, data of intervention studies are lacking. Only in one very small trial lipoprotein (a) was lowered by extracorporeal elimination without showing an additional positive effect on coronary heart disease. Yet, as no levels below 30 mg/dl could be obtained, the results of this study warrant further investigation.

Apolipoprotein (a) contains multiple repeated kringle domains that are similar to a sequence found in plasminogen. Differing numbers of kringle sequences in apolipoprotein (a) give rise to lipoprotein (a) isoform size heterogeneity. In addition to elevated lipoprotein (a) plasma concentration apolipoprotein (a) isoform size has been discussed as a risk factor for coronary heart disease, but this could not be shown in all studies.

A problem which has to be solved in the future is the enormous interassay variance of lipoprotein (a) determination especially at high concentrations. Until this problem has been solved, follow-up data have to be obtained by the same assay with the same calibration.

### Lipoprotein (a) concentration in HIV-infected patients

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Lipoprotein (a) is thought to be involved in the pathogenesis of atherosclerosis. An elevated lipoprotein (a) above 70 mg/dl or elevated lipoprotein (a) above 30 mg/dl with concomitantly elevated LDL-cholesterol  $> 160$  mg/dl seem to be risk factors for cardiovascular disease. We discuss the following hypotheses: I. In a significant subset of HIV infected adults and children lipoprotein (a) is elevated and this correlates with antiretroviral therapy. II. In HIV negative individuals with coronary heart disease lipoprotein (a) is elevated to a similar degree as in HIV positive individuals. Published as well as new data from HIV infected adults and children are reviewed.

I. In a group of 300 HIV-positive adults followed at a HIV clinic in Stuttgart (Germany) (266 men, 34 women, mean age  $40.4 \pm 9.2$  years, 245 treated with antiretroviral drugs) median lipoprotein (a) was 13 mg/dl (interquartile range 4 – 48 mg/dl). 59 patients (19,6%) had either lipoprotein (a)  $> 70$  mg/dl or  $> 30$  mg/dl plus an elevated LDL-cholesterol  $> 160$  mg/dl. 104 patients (34,6 %) had a lipoprotein (a)  $> 30$  mg/dl. Similarly, the prevalence of lipoprotein (a)  $> 30$  mg/dl in other smaller cohorts was 22 – 33 %. In 53 children on an antiretroviral therapy median lipoprotein (a) was 21 mg/dl (interquartile range 8.0 – 55 mg/dl), 12 of them (22 %) had a lipoprotein  $> 70$  mg/dl and two (4 %) above 30 mg/dl plus LDL-cholesterol above 160 mg/dl and 8 (15 %) above 30 mg/dl plus LDL-cholesterol  $> 110$  mg/dl.

HIV-infected patients who required no antiretroviral therapy ( $n = 55$ , Stuttgart cohort) were compared to treated patients ( $n = 245$ ). Patients from a different cohort (Swiss Cohort,  $n = 65$ ) who required antiretroviral treatment had a much lower median lipoprotein (a) concentration. 14 out of 65 (22 %) patients had lipoprotein (a) above 30 mg/dl (three above 70 mg/dl (5 %)), LDL-cholesterol was  $< 100$  mg/dl in all patients. 75 HIV-infected patients in the Stuttgart and Duesseldorf cohorts were followed longitudinally up to two years. After initiating HAART only about half of the patients showed an increase of lipoprotein (a). No association was found with any type of drug therapy, neither NRTI nor NNRTI or protease inhibitors. From this data hypothesis I can only partly be accepted: A significant number (about one fifth) of HIV-infected patients do have an increased risk for coronary heart disease, but this seems to be independent of antiretroviral therapy.

II. We then compared the above data from HIV positive patients with data from HIV negative patients (Nuremberg Cohort, patients from a cardiology center) with or without coronary heart disease. Median lipoprotein (a) was 17 mg/dl in patients without coronary heart disease (interquartile range 8.0 – 46.5 mg/dl,  $n = 200$ ). 44 patients (22 %) had a lipoprotein (a) above 70 mg/dl or above 30 mg/dl plus LDL-cholesterol  $> 160$  mg/dl. Median lipoprotein (a) was 22.5 mg/dl in patients with coronary heart disease (interquartile range 9.0 – 61.5 mg/dl,  $n = 154$ ), 39 patients (25 %) had lipoprotein (a) above 70 mg/dl or above 30 mg/dl plus LDL-cholesterol  $> 160$  mg/dl. 80 % of the patients with lipoprotein (a) above 30 mg/dl were treated with lipid-lowering drugs. While median lipoprotein (a) levels may not indicate risk for coronary heart disease it is helpful to focus on very high lipoprotein (a) levels: 22 % with coronary heart disease had lipoprotein (a)  $> 70$  mg/dl compared to 5 % without coronary heart disease.

In summary, a substantial part of HIV-infected patients is at increased risk for coronary heart disease due to elevated lipoprotein (a). Concentrations are not higher than in a normal population and appear to be lower as compared to a non-HIV-infected cohort with coronary heart disease. The value of lipoprotein (a) as a risk factor for cardiovascular disease in HIV-infected patients needs to be evaluated in prospective cohort trials.

### Importance of non-lipid risk factors for coronary heart disease in HIV-infected patients

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#### *I. Differences due to acquisition of HIV-infection*

Cardiovascular risk factors of 309 HIV-infected adults (HIV-acquisition: 59.2 % by homosexual contact (group 1), 28.5 % by heterosexual contact (group 2), 9.1 % by intravenous drug abuse (group 3) and 3.2 % by blood transfusion (group 4)) were analysed. Overall 10-years probability for cardiovascular events was analysed by the Framingham algorithm.

*Results:* Tobacco use was more common in group 1 subjects compared with group 2 subjects (67 % vs. 52 %;  $p < 0.05$ ). Additionally, group 1 subjects exhibited elevated total cholesterol ( $5.6 \pm 0.1$  mmol/l vs.  $4.8 \pm 0.3$ ), LDL-cholesterol ( $3.6 \pm 0.1$  mmol/l vs.  $2.8 \pm 0.2$ ) and triglyceride concentrations ( $3.2 \pm 0.3$



mmol/l vs.  $1.7 \pm 0.2$ ) compared with group 3 (all  $p < 0.05$ ). No significant differences between the groups were detected in glucose metabolism. The overall 10-years probability for cardiovascular events was significantly higher in group 1 compared with group 2 and group 3 ( $12.2 \pm 0.8\%$  vs.  $6.6 \pm 0.9\%$  and  $7.9 \pm 1.6\%$ ,  $p < 0.05$ ).

**Conclusions:** The cardiovascular risk profile differs between subgroups of HIV-infected individuals, leading to significant higher probability of cardiovascular events in group 1 subjects. The risk of premature atherosclerosis by HIV-infected individuals and therapeutic options remains to be established.

## II. Gender differences

Cardiovascular risk factors of 309 HIV-infected adults, including 240 males were analysed. Overall 10-years probability for cardiovascular events was evaluated by the Framingham algorithm.

**Results:** Gender differences were detected in cardiovascular risk factors such as lipid values, blood pressure and the rate of smoking. Tobacco use was much more common in HIV-infected males compared with HIV-infected females ( $67.5\%$  vs.  $49.3\%$ ;  $p < 0.001$ ). Although no significant difference was noticed in total cholesterol ( $5.49 \pm 0.09$  vs.  $5.53 \pm 0.19$  mmol/l,  $p = 0.84$ ), the HDL-cholesterol concentration was significantly lower ( $1.09 \pm 0.03$  vs.  $1.36 \pm 0.06$  mmol/l,  $p < 0.001$ ) and the triglyceride concentration higher ( $3.01 \pm 0.21$  vs.  $2.06 \pm 0.26$  mmol/l,  $p = 0.02$ ) in HIV-infected males compared to HIV-infected females. Additionally, systolic blood pressure was higher in HIV-infected males compared with HIV-infected females ( $123.1 \pm 1.1$  vs.  $115.4 \pm 2.1$  mmHg,  $p < 0.01$ ). No significant differences were detected in HbA1c concentrations between both groups ( $5.15 \pm 0.07\%$  vs.  $5.31 \pm 0.11$ ,  $p = 0.26$ ). The overall 10-years probability for cardiovascular events was  $8.7\%$  (median) in HIV-infected males and  $1.7\%$  in HIV-infected females ( $p < 0.0001$ ).

**Conclusions:** In the present study, we observed gender differences in the cardiovascular risk profile of HIV-infected individuals. The risk of premature atherosclerosis and associated cardiovascular events was significantly higher in HIV-infected males.

## III. Age differences

309 HIV-infected adults were analysed. Patients were divided into four groups: 18 - 30 years (group 1), 31 - 40 years (group 2), 41 - 50 years (group 3), > 50 years (group 4). Overall 10-years probability for cardiovascular events was evaluated by the Framingham algorithm.

**Results:** Differences between the groups were detected in cardiovascular risk factors including changes in lipid- and glucose metabolism. Lipid values increased with elevated age, such as total cholesterol concentration (Mean  $\pm$  SEM in group 1 vs. group 4:  $4.71 \pm 0.20$  to  $6.36 \pm 0.21$  mmol/l,  $p < 0.05$ ), LDL-cholesterol concentration ( $2.86 \pm 0.17$  vs.  $4.17 \pm 0.21$  mmol/l,  $p < 0.05$ ) and triglyceride concentration ( $1.56 \pm 0.14$  vs.  $3.48 \pm 0.40$  mmol/l,  $p < 0.05$ ). HDL-cholesterol concentration did not show a significant difference ( $1.15 \pm 0.03$  mmol/l). Glucose concentration increased with elevated age in HIV-infected patients ( $5.28 \pm 0.19$  vs.  $6.46 \pm 0.24$  mmol/l,  $p < 0.05$ ), but there was no significant difference in HbA1c - concentration, blood pressure and smoking rate between the groups. The overall 10-years probability for cardiovascular events was higher in group 1 (median:  $1.9\%$ ) than in group 4 ( $20.5\%$ ;  $p < 0.01$ ).

**Conclusions:** The risk of cardiovascular events is related to the age in HIV-infected patients. Therefore, an increased duration of life due to a more effective antiretroviral therapy will have a significant impact on the rate of cardiovascular events in this pa-

tient population. In the future, further increase of cardiovascular events in HIV-infected patients may be expected.

## Impact of lipodystrophy on lipids in HIV-infected patients

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Lipodystrophy in HIV-1-infected patients represents an adverse effect of antiretroviral therapy, not limited to a specific drug or class of drugs. It is not completely known whether lipodystrophy is one unique syndrome or several different overlapping syndromes. Metabolic features such as increased levels of triglycerides, total, LDL- and VLDL-cholesterol, reduced HDL-cholesterol, insulin resistance, and body fat abnormalities consisting of a lipodystrophy-generalized decrease of subcutaneous fat with or without lipohypertrophy, intra-abdominal, breast or dorso-cervical accumulation have been commonly reported, although the intensity and the associations of those changes among themselves have been highly variable.

Metabolic alterations observed in HIV-infected patients might be either a consequence of HAART, HIV infection, or body fat changes, or the combination of all three. Potential exclusive impact of body fat abnormalities on lipids in HIV-infected patients is therefore hard to be elucidated.

Only recent studies have indicated that adipose tissue is not a passive site of energy storage but is also an endocrine organ with broad secretory activity of adipocytokines such as TNF- $\alpha$ , IL-6, IL-8, adiponectin, leptin and resistin. Adipocytokines are bioactive peptides that may play an important role in the regulation of glucose and lipid metabolism.

Dysfunction of fat cell differentiation and increased apoptosis observed in HIV-infected patients with lipodystrophy may lead to defective endocrine function of adipose tissue. Increased expression of TNF- $\alpha$ , IL-6 and IL-8, and decreased levels of adiponectin and leptin have so far been described.

While leptin levels are related to subcutaneous fat content and the level of adiposity, and adiponectin levels are associated with measures of fat distribution, both leptin and adiponectin may play an important role in metabolic abnormalities and both are altered in HIV lipodystrophy syndrome.

Plasma adiponectin and leptin concentrations were inversely and directly associated with plasma Apo B-48, Apo C-III, HDL-cholesterol, triglycerides, VLDL-Apo B, and VLDL-triglycerides. These data suggest that plasma adiponectin concentration may not only link abdominal fat, insulin resistance, and dyslipidemia, but may also exert an independent role in regulating concentration of free fatty acids and triglycerides metabolism. Since it is stimulated by insulin and inhibited by TNF-alpha, insulin resistance and TNF-alpha expression may contribute to this effect.

HIV-lipodystrophy syndrome is associated with an accelerated rate of lipolysis, increased release of free fatty acids that results in increased hepatic fatty acid re-esterification to triglycerides, and impaired triglyceride storage could lead to hypertriglyceridemia and decrease of HDL-cholesterol. Some clinical trials, however, failed to show this dependence.

Other derangements have been described, although their relationship with the lipodystrophy syndrome and its impact on metabolic alteration has not been clearly established. Knowledge of

some aspects of this problem has increased in recent years, but many important questions remain to be solved in this cascade of events.

### Accumulation of dorsocervical fat ("buffalo hump") in HIV-infected patients.

#### A case control study (LIPOCER STUDY)

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#### Objectives:

Lipodystrophy is very common in HIV-patients on HAART. However, buffalo hump (BH) is only reported in 2 - 13% of HIV-infected patients. Our aim was to analyse the prevalence of BH in HIV-infected patients on HAART and the factors associated with its development.

#### Methods:

Multicenter, observational, 1:1 case-control study of HIV outpatients from 10 Spanish institutions. Cases: HIV patients with dorsocervical fat accumulation. Controls: HIV patients from the same cohort, controlled by age ( $\pm 5$  years), sex, and BMI ( $\pm 2.5$  kg/m<sup>2</sup>). Epidemiological, clinical, and analytical data were collected. Statistic program: SPSS® 10.0.

#### Results:

A BH was reported in 80 (1.8%) patients from a cohort of 4,214 patients on HAART followed up in the participant institutions. Four of the patients with a BH were excluded as no matched controls could be found. Those patients with a BH had a longer evolution of HIV infection (139 vs. 107 months;  $p = 0.0001$ ), more exposure to PI (91 vs. 68 %;  $p = 0.002$ ), EFV (60 vs. 40 %;  $p = 0.032$ ), and d4T (91 vs. 54 %;  $p = 0.0001$ ), higher waist-to-hip ratio (0.98 vs. 0.93;  $p = 0.0001$ ), triglyceride levels (230 vs. 156 mg/dl;  $p = 0.0001$ ), and CD4 cell count (622 vs. 513/ $\mu$ l;  $p = 0.021$ ), and lower HDL-cholesterol levels (43 vs. 52 mg/dl;  $p = 0.001$ ); they were more likely to have lipoatrophy (83 vs. 33 %;  $p = 0.0001$ ), gynaecomasty (16 vs. 1 %;  $p = 0.005$ ), neuropathy (8 vs. 0%;  $p = 0.028$ ), and prior metabolic disorders (53 vs. 18 %;  $p = 0.0001$ ). In the multivariate analyses, only increasing time of exposure to d4T (for each 6 months increase: 5.82, 95 % CI 5.70 - 5.94;  $p = 0.0073$ ), and lipoatrophy (8.04, 95 % CI 2.93 - 22.02;  $p = 0.00001$ ) were independently associated with a BH. Conclusions: Although lipodystrophy is very frequent among HIV-infected patients on HAART, BH is an uncommon kind of fat redistribution in this population. It was related to time of exposure to d4T and lipoatrophy.

### Metabolic abnormalities and lipodystrophy syndrome in HIV-infected children in Poland

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Metabolic abnormalities and clinical signs of lipodystrophy are known adverse effects of antiretroviral therapy in HIV infected children. The consequence may be early atherosclerosis e. g. ischaemic heart disease.

#### Aim:

Estimation of prevalence of metabolic abnormalities and lipodystrophy in HIV infected children receiving Highly Active Antiretroviral Therapy (HAART).

#### Patients and methods:

57 children (aged 10 months - 17 years) were included into the study. The patients history was documented and physical examination was done every 12 weeks. Laboratory tests included: triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, and glucose. In 41/48 patients body composition was measured twice by Bioelectrical Impedance Analysis (BIA). Patients were divided into 2 groups: 1) below 3 years of age (mean 24 months) – 9 children; 2) above 3 years (mean 8 years and 9 months) – 48 children. 3 years of age was estimated to be the border for the development of lipodystrophic signs.

#### Results:

Metabolic abnormalities were observed in 54/57 children: in all children < 3 years and in 45/48 of older children. There were increased levels of triglycerides in 50 cases, of cholesterol in 45 cases and low HDL-cholesterol in 14 cases.

Lipodystrophy was found in 15/48 children > 3 years (60% girls and 40% boys). All received HAART (mean time: 4 years and 8 months), their mean age was 9 years and 3 months; 5/15 had AIDS; 5/15 had severe immunodeficiency. The characteristics of the group without lipodystrophy (33/48) were: 60.6% boys, 39.4% girls, mean age 8 years and seven months; 9/33 had AIDS; 14/33 severe immunodeficiency. Family history was burdening in 60% cases in both groups. Metabolic abnormalities were observed in all children with lipodystrophy and 90,9% without lipodystrophy. All children with lipodystrophy were treated with stavudine, often stavudine and didanosine. Of the children with lipodystrophy 6 had lipoatrophy (5 boys, 1 girl), one girl had lipohypertrophy, 9 mixed forms (8 girls, 1 boy); of 13/15 children examined with BIA 23% had normal, 23% decreased and 54% increased fat mass in comparison with 28/33 girls without lipodystrophy (29%, 7%, and 64%).

#### Conclusions:

Metabolic abnormalities in HAART treated children are frequent (95%). Advance of disease and time of HAART treatment (especially with stavudine and didanosine) increase risk for this syndrome.

## Extended follow-up of cardiovascular and cerebrovascular outcomes and deaths in a retrospective cohort of HIV patients treated in US Veterans Affairs Hospitals

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Metabolic abnormalities associated with human immunodeficiency virus (HIV) infection, including dysglycemia and hyperlipidemia, are increasingly prevalent, and there is growing concern about associations between treatment, lipids and the risk of accelerated cardiovascular and cerebrovascular disease. Our group previously published an outcomes study that reported on cardiovascular/cerebrovascular outcomes among HIV treated individuals in the Veterans Administration system (Bozzette et al. *N Engl J Med.* 2003 Feb 20; 348(8): 702-10). We used administrative data to study of the risk of cardiovascular and cerebrovascular disease and death among the 36,766 patients who received care for HIV infection at US Veterans Affairs facilities between January 1993 and June 2001.

For antiretroviral therapy, 70.2 percent of the patients received nucleoside analogues, 41.6 percent received protease inhibitors, and 25.6 percent received nonnucleoside reverse-transcriptase inhibitors for a median of 17 months, 16 months, and 9 months, respectively. Patient-level regression analyses indicated that there was no relation between the use of nucleoside analogues, protease inhibitors, or nonnucleoside reverse-transcriptase inhibitors and the hazard of cardiovascular or cerebrovascular events, but the use of antiretroviral drugs was associated with a decreased hazard of death from any cause.

We concluded that the use of newer therapies for HIV was associated with a large benefit in terms of mortality that was not substantially diminished by any increase in the rate of cardiovascular or cerebrovascular events or related mortality. Shortly thereafter, the carefully conducted DAD study used a prospective pseudo-cohort design to show similar overall benefits to treatment but an increased risk of cardiovascular events. Both groups conclude that prolonged survival among HIV-infected patients meant that longer-term observation in the clinical setting and in the prospective cohort study were indicated.

We have since repeated and somewhat extended this analysis on an updated VA cohort. The expanded cohort includes 41,213 patients and extends follow-up from 121,936 to 168,611 patient-years. The number of patients having at least 4 years of exposure to any ARV increased from 4,808 in the original cohort to 8,582 in the 1993-2003 cohort. There were 1,735 cardiovascular admission and 17,558 deaths in the updated cohort. Rates of cardiovascular admissions were steady at about 1 per 100 patient-years while death rates continued to decline to a low of 5.2 per 100 patient-years in 2003. Differences between cumulative survival and cumulative survival with cardiovascular disease continued to be trivial. However, preliminary modeling may suggest that trends of interesting the risk of cardiovascular disease may be emerging.

## Hospitalizations for Coronary Heart Disease and Myocardial Infarction Among Northern California Men With and Without HIV-1 Infection

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### Background:

In our US population of HMO-enrolled HIV-1 infected patients we have reported increased risk of CHD and MI compared to the base population of HIV-1 uninfected members. We have also examined the differential risk of these events in patients exposed vs. unexposed to the protease inhibitor (PI) class of antiretroviral agents. We have found increased risk with exposure that appears to increase with duration of exposure, however with less certainty than other researchers. The documented atherogenic lipid changes consequent to PI therapy for HIV-1 infection contribute to CHD/MI risk. Our current work updates previous risk estimates as exposure time and events accumulate. We also describe how changes in medical practice might explain the relatively muted effect of PI exposure and an apparent recent reduction in CHD and MI event rates despite increased duration of PI exposure.

### Methods:

We continue to monitor and compare hospitalization rates for CHD (ICD9 410-414) and separately for MI (ICD9 410) among a cohort of HIV(+) males and among a random sample of presumed HIV(-) males. Persons studied were members of the Kaiser Permanente Northern California health plan, a large closed system HMO, and had no prior CHD events. Observational follow-up (FU) began in 1996 and now extends through 6/30/05. Among HIV+'s, person-years (PY) of FU was assigned as either no/pre-PI exposure vs. during/post PI-exposure. A person could contribute PY to both exposure categories. Age-adjusted CHD and MI rates for ages 35-64 were calculated overall for HIV+'s and HIV-'s, and by PI exposure and duration of PI exposure for HIV+'s. Traditional risk factors, antiretroviral prescriptions and lipid measurements were assessed from electronic medical records.

### Results:

In the 9.5-year observation period, 5430 HIV+ patients contributed 26,882 PY of FU (median 4.6) and there were 140 CHD events (86 MIs). Over forty thousand HIV-'s contributed 307K PY of FU. Among HIV+'s exposed to PIs, median PI exposure was 4.3 years. Age-adjusted CHD and MI rates among HIV+'s continue to be roughly twice that of HIV-'s (CHD: 6.0 vs. 2.9 events/1000 PY,  $p < 0.0001$ ; MI: 3.6 vs. 2.2,  $p = 0.002$ ). Among HIV+'s, overall rates for never vs. ever PI exposure were not significantly different but were suggestive of an effect (CHD: 4.8 vs. 6.9,  $p = 0.09$ ; MI: 3.0 vs. 4.2,  $p = 0.20$ ). The age-adjusted relative risk for CHD hospitalization per two years of PI exposure was 1.05 (95% CI: 0.89, 1.30,  $p = 0.22$ ); relative risk for MI was 1.16 (1.0, 1.4,  $p = 0.11$ ).

### Conclusions:

Even in the absence of PIs, HIV(+) persons have twice the rate of hospital CHD and MI as their HIV(-) counterparts. Traditional risks, including the dyslipidemia associated with HIV infection (low HDL), as well as chronic inflammation are a likely basis for this observation. We are not seeing a worsening of the apparent initial rise in the number and rate of CHD and MI events. This is possibly due to increased use of 'lipid-friendly' antiretroviral regimens and greater attention to coronary risk in HIV-infected individuals.

**Relationship between prolonged exposure to combination antiretroviral therapy (cART) and myocardial infarction (MI): effect of sex, age and lipid changes. Results from the D:A:D Study.**

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*Background:*

Combination antiretroviral therapy (cART) for the treatment of HIV-1 infection can induce metabolic adverse effects, including dyslipidemia and insulin resistance, which confers potential risk for cardiovascular disease (CVD). Prior data from the D:A:D Study suggest an association of cART exposure with an increased risk of MI. We investigated if the linear trend previously reported continues with further cART exposure, whether it is modified by age or sex, and possible mechanisms for this relationship.

*Methods:*

D:A:D is a prospective multi-cohort study investigating the incidence of CVD and factors that affect this. 23,437 HIV-infected persons (24% women) from 188 clinics in Europe, USA and Australia were enrolled from 1999 to 2001, and followed prospectively thereafter. Results based on follow-up to February 2004. Incidence rates of first prospective MI (/1000 person-years (PY)), and relative rates (RR) of factors associated with MI from Poisson regression models are reported.

*Results:*

At present the study population has contributed more than 76,500 person years of follow-up (PY). During this time, 277 patients experienced a first MI. The MI incidence increased from 1.39/1000 PY in those not exposed to cART, to 6.07/1000 PY in those exposed for >6 years (RR compared to no exposure: 4.38 [95% CI: 2.39 - 8.04],  $p = 0.0001$ ). After adjustment for other potential risk factors, there was a 1.17 fold

[1.11 - 1.24] increased risk of MI per additional year of cART exposure. Similar results were found after inclusion of repeat MIs (1.18 [1.09 - 1.28]), inclusion of only definite MI (58.5 % of MIs) (1.22 [1.11 - 1.35]), and restricting the analysis to naïve patients ( $n = 4161$ ) at entry (1.39 [0.93 - 2.08]).

Although the absolute rate of MI was higher in men than women (2.04 [1.30 - 3.21]), the RR associated with cART was similar in men (1.14 [1.06 - 1.24]) and women (1.38 [1.07 - 1.76],  $p$ -value for interaction 0.51). The relationship was similar in younger and older patients (men > 45 and women > 55 years;  $p$ -value for interaction 0.41). Including time-updated levels of serum total cholesterol (RR 1.15 [1.06 - 1.25] per mmol/l), HDL-cholesterol (0.60 [0.42 - 0.88] per mmol/l), and triglycerides (1.64 [0.98 - 2.74] per log<sub>2</sub>) in the same model, reduced the association of additional year of cART with MI to 1.10 [1.01 - 1.19]. Adjustment for lipid lowering medication did not further affect the association between cART exposure and MI.

*Conclusions:*

These findings suggest that while the overall absolute risk of MI remains modest in this relatively young population, the risk continues to increase with longer exposure to cART over the first 7 years of use. Dyslipidemia explained part but not all of the association of cART with risk of MI. Conversely, our data do not suggest any association with level of immune deficiency, HIV-RNA or duration of HIV-infection [data not shown]. The relative increase in risk appears similar in men and women, and in older and younger subjects. Based on the current evidence, clinicians are encouraged to carefully monitor the risk of CVD in HIV-patients receiving cART, and to intervene according to standard care.

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