Humana Press

Cardiovascular Toxicology (2004) 04 303–315 $25.00 (http://www.cardiotox.com)
 1530-7905/01

HIV, Metabolic Syndrome X, Inflammation, Oxidative Stress, and Coronary Heart Disease Risk

Role of Protease Inhibitor Exposure

Barry E. Hurwitz,[1,2,3,*] Nancy G. Klimas,[1,5] Maria M. Llabre,[1,2] Kevin J. Maher,[1,5] Jay S. Skyler,[1,3] Martin S. Bilsker,[1,4] Shvawn McPherson-Baker,[1,5] Peter J. Lawrence,[1] Arthur R. LaPerriere,[1,6] Jeffrey M. Greeson,[2] Johanna R. Klaus,[2] Rasha Lawrence,[1] and Neil Schneiderman[1,2,3,6]

[1]Behavioral Medicine Research Center, University of Miami, Miami, FL; [2]Department of Psychology, University of Miami, Coral Gables, FL; Divisions of [3]Endocrinology and Metabolism, [4]Cardiology, Department of Medicine, University of Miami, Miami, FL; and Departments of [5]Microbiology and Immunology, [6]Psychiatry and Behavioral Sciences, University of Miami, Miami, FL

Abstract

Differences on measures of metabolic syndrome X and coronary heart disease (CHD) risk, as well as potential pathophysiological mediators, inflammation, and oxidative stress, were examined as a function of HIV serostatus and highly active antiretroviral therapy (HAART) regimen with and without protease inhibitors (PIs). Data from 164 men and women, aged 18 to 55 yr, were used to compare 82 HIV$^+$ subjects who were free of hepatitis C virus and were on a stable HAART regimen for ≥6 mo, with 82 seronegative subjects matched on age, sex, body mass index, and ethnicity. For the HIV$^+$ subjects, after controlling for diabetes status and HIV disease progression, PI exposure was associated with greater oxidative stress, triglyceridemia, and lipidemia than it was for non-PI-exposed HIV$^+$ subjects, and the risk of a future myocardial infarction was up to 56% greater in PI-exposed than in non-PI-exposed subjects and 129% greater than in controls. Although it is likely that the greatest proportion of CHD risk in the HIV$^+$ subjects may be accounted for by pathological conditions linked to HIV infection in interaction with mediating processes such as inflammation, central obesity, and dyslipidemia, which was greater than in controls, it appears that PI medications may exacerbate oxidative stress and hypertriglyceridemia to enhance this risk.

Key Words: HIV/AIDS; HAART; coronary heart disease risk; metabolic syndrome X; inflammation; oxidative stress.

*Author to whom all correspondence and reprint requests should be addressed: Barry E. Hurwitz, PhD, Behavioral Medicine Research Center, (200 BMRC), c/o VA Medical Center, 1201 N.W. 16th Street, Miami, FL 33125. E-mail: bhurwitz@miami.edu

Received: 1/23/2004

Revised: 3/2/2004

Accepted: 3/9/2004

Cardiovascular Toxicology, vol. 4, no. 3, 303–315, 2004

Introduction

Studies of HIV-seropositive (HIV^+) persons have linked highly active antiretroviral therapy (HAART) regimens including protease inhibitors (PIs) with lipodystrophy and elevated circulating levels of triglycerides, cholesterol, insulin, and fasting glucose *(1–3)*. These findings have stimulated interest in the role of specific anti-HIV medications and the mechanisms by which HAART may induce these cardiovascular complications. The metabolic syndrome X (MSX) comprises a constellation of comorbid alterations affecting central fat deposition, glucose tolerance, cardiac structure and function, and vascular endothelial function, and includes dyslipidemia, hypertriglyceridemia, and insulin resistance; the syndrome confers greater atherosclerotic risk than each factor does independently *(4,5)*. The combined influences of resistance to insulin-mediated glucose uptake (i.e., diminished insulin sensitivity), hyperinsulinemia, and sympathetic activation have been posited to operate in a positive feedback loop to promote atherosclerosis *(5)*. Features of MSX have been estimated to appear in up to 60% of the HIV^+ patients treated with HAART *(6,7)*.

One recent study has shown that the degree of insulin resistance in HIV^+ persons is associated with the levels of tumor necrosis factor (TNF-α), a proinflammatory cytokine, suggesting that inflammatory and metabolic mechanisms are linked pathophysiological processes *(8)*. It is now widely accepted that atherosclerosis is the result of a prolonged and excessive inflammatory process in the vascular wall *(9)*. Proinflammatory immune cytokines (i.e., interleukin [IL]-1, IL-6, TNF-α) are released by injured or jeopardized tissue, by macrophage, and in response to invading pathogens, but are released in greatest quantity by adipose tissues *(10,11)*. These cytokines also stimulate the production of reactive oxygen species *(12)*. The excessive formation of free radicals when in imbalance with antioxidant capacity is termed oxidative stress. Free radicals, the consequence of oxidative metabolism, are highly reactive because these molecules contain an unpaired electron. As a result, these molecules can damage essential biological molecules, such as fats, proteins, and DNA *(13)*. In addition to inflammation, the oxidative modification of low-density lipoprotein-cholesterol (LDL-C) by liperoxide products leads to enhanced uptake by macrophage and increased cellular accumulation of cholesterol-loaded foam cells and the generation of fatty streaks, an early step in the formation of plaque in the vessel walls *(13)*. The proinflammatory cytokines also promote the infiltration of phagocytes and inflammatory leukocytes into the liver and heart *(14)*. In addition, they stimulate hepatic synthesis of C-reactive protein (CRP), which has been used as an index of the extent of inflammatory activity *(15)*. After correcting for traditional risk factors, CRP is a strong independent predictor of future cardiovascular events in healthy men, postmenopausal women, and the middle-aged and elderly, as well as in those with preexisting angina *(16,17)*.

Although in the last few years the literature has begun to assess whether certain HAART regimens with or without PIs differentially influence cardiovascular disease risk, the evidence has not consistently demonstrated the extent to which PI exposure is associated with the features of MSX in HIV spectrum disease (*see* review in ref. *18*). The literature assessing HAART impact on cardiovascular disease risk suffers from numerous design problems (e.g., small sample sizes, nonobjective and nonvalidated case definitions) and inconsistently addresses possible confounds (e.g., heterogeneity of HIV populations, cardiovascular disease risk profile, concomitant treatments for diagnosed cardiovascular conditions or medications with cardiovascular effects, illicit drug use, immune status, anti-HIV treatment duration, time to immune stabilization following initiation of anti-HIV medication regimen) or lacks comparison of HIV^+ participants with appropriately matched seronegative persons. Findings of HAART-related cardiovascular complications in longitudinal studies of anti-HIV medication-naive patients are difficult to evaluate because of heterogeneous sample selection based on recency of diagnosis and disease history. Moreover, infection with hepatitis C virus (HCV) is another potential confounding influence to which the literature has paid scant attention; HCV infection independent of HIV infection confers increased MSX risk *(19)* and HCV coinfection in HIV^+ persons is highly prevalent *(20)*.

Therefore, this study assessed the influence of HAART treatment with and without PIs to determine (1) the extent to which HIV serostatus, independent

of HCV coinfection, exacerbates MSX and risk of a future acute coronary event; and (2) whether differences in measures of proinflammatory and oxidative stress processes may be playing an essential mediational role in exacerbating cardiovascular disease pathophysiology. This study controlled for potential confounding influences by comparing HIV-seropositive persons with HIV-seronegative persons matched on age, sex, body mass index (BMI), and ethnicity.

Materials and Methods

Subjects

The HIV$^+$ subjects were participants in the Miami Selenium and Heart Health clinical trial. This trial was designed to determine whether supplementation with selenium, an antioxidant mineral, would influence cardiovascular and immune functioning in HIV$^+$ men and women, with and without a history of cocaine use. Subjects were recruited through outpatient services within the University of Miami–Miami Veterans Administration Medical Center complex and through local HIV support service agencies, private physician referrals, and newspaper advertisements. Subjects were included if they (1) provided informed consent; (2) presented documented evidence of their HIV-1 infection; (3) were 18–55 yr of age; (4) were not being treated pharmacologically for a diagnosed cardiovascular condition (e.g., beta-blockers, calcium antagonists, angiotensin-converting enzyme inhibitors), for carbohydrate conditions (e.g., hypoglycemics, insulin sensitizers), for psychiatric conditions (e.g., antipsychotics), and for endocrine conditions (e.g., estrogen hormonal replacement); (5) had no history of hypertension or other cardiorespiratory disorder diagnosis; (6) were HCV seropositive and not treated for this infection within 6 mo prior to study entry; (7) were not being treated with HAART medication, or had been treated with one stable and well-tolerated HAART medication regimen for ≥6 mo; (8) were not pregnant; (9) presented no evidence of myocardial infarction or atrioventricular (AV) conduction arrhythmias on electrocardiogram (ECG) examination; and (10) did not have chronic illness associated with immune alterations or a recent (<3 mo) acute infection or surgery.

One hundred and fifty HIV$^+$ men and women enrolled and completed the procedures. This sample comprised individuals spanning several exposure categories (e.g., homosexual/bisexual, heterosexual, intravenous drug use [IVDU] history) and manifesting various HIV infection stages (HIV asymptomatic, HIV symptomatic, AIDS). Of these individuals, 82 subjects had been treated for ≥6 mo on one stable and well-tolerated HAART medication regimen that included some combination of PI, nucleoside reverse transcriptase inhibitor (NRTI), nucleotide reverse transcriptase inhibitor (NtRTI), and nonnucleoside reverse transcriptase inhibitor (NNRTI). The remainder of the subjects were not treated with anti-HIV medications ($n = 23$), were treated for <6 mo on HAART ($n = 4$), or were positive for HCV coinfection on ELISA and confirmed with RNA PCR assay ($n = 41$) and hence were excluded from the study analyses. According to the 1993 Centers for Disease Control classification system (using self-reported historical low CD4 count), of these 82 subjects, 18 were asymptomatic, 6 were symptomatic but not AIDS defined, and 58 were AIDS defined *(21)*. Other demographic and related characteristics of this HIV-seropositive sample are presented in Table 1. As can be seen from Table 1, 50% or 41 of the HIV$^+$ subjects were on HAART regimens including PIs, and the remaining 50% or 41 of these subjects were on HAART regimens without PIs. The ethnicity and sex composition of the sample was similar to that present in South Florida *(22)* and in our previous samples *(23)*.

The 82 HIV$^+$ subjects were matched one by one with 82 HIV-seronegative (HIV$^-$) subjects on the basis of age, sex, BMI, and ethnicity. Respective mean ± SD difference between HIV groups for age and BMI was 0.25 ± 2.9 yr and 0.37 ± 2.6 kg/m^2. No difference on sex or ethnicity composition was present. The HIV$^-$ subjects were selected from 329 persons who participated in the Markers Assessing Risk for Cardiovascular Health study, designed to assess preclinical cardiovascular disease risk in relation to MSX indices. All of these subjects were aged 18 to 55 yr and healthy based on physical examination by physician, medical history, fasting blood chemistry analysis, and 12-lead ECG. They had no history of cardiovascular disease or other systemic disorder and were taking no prescribed medication. The procedures used to collect these data were almost identical to the presently used procedures except that the immune measures were not performed.

Table 1
Characteristics of the HAART Groups and the HIV-Seronegative Group

Measure[a,b]	HIV^+PI^+ (n = 41)	HIV^+PI^- (n = 41)	HIV^- Control (n = 82)	Group differences
Age (yr)	40.6 ± 0.9	39.5 ± 1.0	39.9 ± 0.7	—
BMI (kg/m^2)	27.6 ± 0.9	27.0 ± 1.0	26.8 ± 0.6	—
Sex (%)				
Men	70.7	61.0	65.9	—
Ethnicity (%)				—
Black	53.7	46.3	39.0	
Hispanic white	26.8	29.3	39.0	
Non-Hispanic white	12.2	12.2	13.4	
Other	7.3	12.2	8.5	
Family annual income ($K)	12.6 ± 2.1	11.5 ± 2.0	19.6 ± 1.7	[c]$HIV^+ < HIV^-$
Education (# grades)	14.8 ± 1.9	14.4 ± 1.9	13.8 ± 0.3	—
Substance use prevalence in preceding 3 mo (%)				
Alcohol	53.7	43.9	41.5	—
Cannabis	19.5	9.8	4.9	[d]$HIV^+ > HIV^-$
Cigarette smoking	48.8	46.3	17.1	[e]$HIV^+ > HIV^-$
Cocaine	7.3	9.8	8.5	—
Sedatives/hypnotics	12.2	0.0	6.1	—
Hallucinogens	9.8	0.0	3.7	—
Opioids	2.4	0.0	3.7	—
Family history of MI (%)	24.4	29.3	20.7	—
Diabetes mellitus (%)	19.5	4.9	0.0	[f]$PI^+ > PI^-$

[a]Values are mean ± SE unless otherwise indicated;
[b]HIV^+PI^+, HIV-infected protease-inhibitor-exposed group; HIV^+PI^-, HIV-infected protease inhibitor nonexposed group; HIV^- control, HIV-seronegative control group; BMI, body mass index; MI, myocardial infarction.
[c]$t(135) = 3.4$, $p < 0.002$.
[d]$\chi^2(1) = 4.4$, $p < 0.04$.
[e]$\chi^2(1) = 17.4$, $p < 0.001$.
[f]$\chi^2(1) = 4.1$, $p < 0.05$.

Experimental Protocol, Procedures, and Physiological Measures

The protocol consisted of two testing sessions on different days. Subjects who met the inclusion criteria following a telephone screening interview were invited to come to the Behavioral Medicine Research Center (BMRC) for session 1. Subjects were instructed in the preassessment consumption requirements to fast from midnight the night before and to abstain from illicit recreational substances, caffeinated or decaffeinated coffee and tea, other caffeine-containing substances, aspirin and other medications, alcohol, and strenuous exercise. In this study a high rate of compliance (95%) with these preassessment instructions was achieved. Noncompliance resulted in rescheduling the appointment within 2 d.

Session 1

On arrival at the BMRC between 0700 and 0800, informed consent was obtained. Compliance with presession restrictions was determined from self-report and urinary toxicology screens performed for illicit drugs and alcohol. Procedures to confirm that the subject met the inclusion criteria were then undertaken. Information regarding current medical conditions and any pre-existing medical history was obtained by physician. Casual sphygmomanometric blood pressure, 12-lead ECG, height, and weight assessments were performed and BMI was calculated. The circumference of the waist at the level of the umbilicus and the circumference of the hips at the level of the greater trochanters was obtained to calculate waist-to-hip ratio (WHR). Urine was tested

with a toxicology screen (i.e., alcohol, barbiturates, benzodiazepines, cannabinoids, LSD, PCP, THC, morphine, and amphetamines), and for women, a urine pregnancy screen was performed. Blood samples were collected for standard blood chemistries, for enumerative immune measures (i.e., CD4, CD8, CD19, and CD56 cells), for HIV viral load, and for determination of HCV serostatus. Substance use/abuse history was assessed by the Structured Clinical Interview for the DSM-IV (SCID-1 Version 2.0). Subjects were then instructed in the requirements for session 2, which were the same as for session 1.

Session 2

On arrival between 0700 and 0800, subjects were urine toxicology screened. Following confirmation of compliance with the presession instructions and insertion of one iv catheter in each forearm, subjects underwent a euglycemic hyperinsulinemia clamp procedure to derive insulin sensitivity. Pharmacologic hyperinsulinemia was achieved by infusion of insulin, wherein the dosage was based on body surface area. The insulin infusate was prepared in 250 mL 0.9% NaCl to which was added 10 mL of the subject's blood (2 mL/50 mL infusate) to prevent absorption of the regular recombinant human insulin (Humulin-R, Eli Lilly, Indianapolis, IN) to glass or plastic surfaces. A 10-min priming infusion was followed by a constant infusion at 40 $\mu U/m^2/min$ for approx 150 min, using a calibrated IMed Gemini PC-2TX infusion pump (Alaris Medical Systems, San Diego, CA). Glucose infusion was begun 4 min after the initiation of insulin infusion and was empirically set at 2.0 mg/kg/min and then increased at 10 min to 2.5–3 mg/kg/min. Blood glucose was clamped to maintain euglycemia within 5% of the fasting value by feedback-controlled infusion of 20% dextrose. To monitor blood glucose concentration and adjust the dextrose infusion, we measured whole blood glucose every 5 min by an enzymatic glucose oxidase method using an YSI 2300 STAT Plus glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). During this steady-state phase, the rate of glucose infusion is equal to the rate of total body glucose uptake and therefore was a measure of tissue insulin sensitivity, labeled "M." Specifically, M was defined as the mean exogenous glucose disposal rate in mg/kg/min by calculating the steady-state glucose infusion rate over consecutive 20-min periods and applying a space correction factor *(24)*.

Blood Assays

Serum cholesterol and triglycerides were measured enzymatically by autoanalyzer (Cobas-Mira Plus, Roche Diagnostics, Branchburg, NJ) using procedures as described *(25,26)*. High-density lipoprotein-cholesterol (HDL-C) was measured after precipitation of apoB-containing lipoproteins with dextran sulfate *(27)*, and LDL cholesterol calculated by the Friedewald method *(28)*. Respectively, intra- and interassay coefficients of variation (CVs) for cholesterol are <2.5 and <3.5%, and for triglycerides are <3.9% and <1.1%. Helper/inducer ($CD3^+CD4^+$) T-cell, suppressor/cytotoxic ($CD3^+CD8^+$) T-cell, B-cell ($CD19^+$), and natural killer cell ($CD3^-CD56^+$) counts were determined using three-color direct immunofluorescence flow cytometry as previously described *(29)*. Intra- and interassay CVs for flow cytometric measures are <5%. HIV viral load was determined using the ultrasensitive Amplicor RT/PCR assay (Roche Diagnostics). For HCV, samples were tested for the presence of HCV antibodies using ELISA (Ortho-Diagnostics, Raritan, NJ). Upon testing positive for the presence of antibodies to HCV, a confirmatory RNA PCR analysis was performed using the COBAS Amplicor HCV MONITOR test v2.0 (Roche Diagnostics, Branchburg, NJ). The index of inflammation, CRP, was measured using a high-sensitivity assay. Diluted serum was incubated with polystyrene particles coated with monoclonal antibodies to CRP to produce agglutination and light scattering in proportion to the concentration of antigen. The serum standard was diluted to produce a standard curve from 0.08 to 6 mg/L. The intra- and interassay CVs for CRP are <4.4% and <5.7%, respectively. Oxidative stress was indexed by measuring plasma 8-epi-prostaglandin-F2α (8-isoprostane) using ELISA methods (Cayman Chemicals, Ann Arbor, MI).[a] The intra- and inter-assay CVs for 8-isoprostane are 11.3% and 10.4%, respectively.

[a]Although there is no generally accepted oxidative stress index, the literature has used indirect parameters, such as thiobarbituric acid reactive compounds (TBARS) *(30)*. More recently, the formation of isoprostanes, such as 8-epi-prostaglandin-$F_{2\alpha}$ (8-isoprostane), is sensi-tive to heightened levels of oxidative stress in various clinical cardiovascular conditions *(31–33)*. Moreover, 8-isoprostane is more applicable to biological samples and is a more specific and sensitive indicator of oxidative stress than other indices, such as TBARS *(34)*.

Coronary Heart Disease Risk

Risk assessment tools based on equations from large epidemiological studies were used to estimate coronary heart disease (CHD) risk. Specifically, the predictive model score and the accompanying probability of sustaining an acute coronary event within 10 yr were calculated using the Framingham *(35)* and PROCAM *(36)* study risk functions. Information regarding age, smoking habit, diabetes status, and systolic blood pressure were used in these methods. Although both methods use HDL-C and LDL-C measures, the PROCAM equation is distinguished by its additional inclusion of family history of myocardial infarction and triglycerides. In addition, the definition of an acute coronary event differs between these risk models. Whereas both the Framingham and PROCAM functions predict fatal and nonfatal myocardial infarction, the Framingham model also predicts incident angina and coronary insufficiency.[b]

Statistical Analysis

The study was a cross-sectional design with nonrandom assignment to groups. The groups of HIV^+ and HIV^- participants were matched as previously described. The HIV^+ participants were further classified on the basis of their HAART regimen (HIV^+PI^+ vs HIV^+PI^-). Hence, planned contrasts within the context of analysis of variance (ANOVA) were performed with the HIV serostatus (HIV^+ vs HIV^-) comparison as one contrast and the HAART regimen comparison as a second contrast on the measures of MSX, inflammation, oxidative stress, and risk of a future coronary event.

The comparison of the HIV serostatus and HAART PI exposure groups revealed no significant differences for most potential control variables (e.g., age, BMI, sex, ethnicity, substance use, education, duration of HAART regimen). When differences on control variables were observed, covariate analyses assessing their potential impact were performed. Values for HIV viral load were normalized using log transformation before analyses. Data were analyzed using SPSS statistical software (SPSS Inc., Chicago, IL).

Results

Group Characteristics

The design yielded three groups: two groups of HIV^+, subjects, with and without PI exposure (HIV^+PI^+, HIV^+PI^-) and their corresponding HIV- matched group (HIV- Control). Table 1 displays the group characteristics. As can be seen in this table, the matching procedures resulted in highly comparable groups, with few differences observed among age, BMI, sex and ethnicity composition, substance use, education, income, and family history of cardiovascular disease. The overall frequency of one or both parents having disease (i.e., myocardial infarction, angina pectoris, hypertension, or stroke) was 45.4%. Significant differences between the groups were observed only in total family income level, cannabis use, and prevalence of smoking. Analyses examining differences between the two HAART-treated groups (HIV^+PI^+ vs HIV^+PI^-) revealed no differences on any of the measures, except that more persons with diagnosed diabetes mellitus were observed in the PI-exposed group.

The comparison of the two HAART groups on immune status, HIV symptom classification, prevalence of HAART medication combinations, and duration on HAART regimen is displayed in Table 2. Measures of immune cell counts were similar between PI-exposed and non-PI-exposed groups, except that the PI-exposed subjects had significantly fewer CD4 cells and a trend toward greater HIV-1 viral load, suggesting that this group was generally at a more progressed point in HIV disease.[c] In addition, PI-exposed subjects were predominantly administered the PI medication in combination with NRTI/NtRTIs, whereas the non-PI exposed were mostly taking the NNRTIs in combination with the NRTI/NtRTIs.

MSX, Inflammation, and Oxidative Stress

Table 3 depicts the mean ±SE values for the measures of MSX, inflammation, and oxidative stress

[b]As per NCEP ATP III guidelines *(37)*, the separate Framingham equations for men and for women were used in this study. However, because the PROCAM function was derived solely from data collected on men, there was only one equation that could be used and as such was applied to both men and women in this study. Therefore, the PROCAM measures of CHD risk derived for the women may be underestimated. A recent comparison of both methods in high-risk men observed that the methods were highly correlated ($r = 0.82$), but the PROCAM method tended to yield greater CHD risk values than the Framingham method when subjects were at higher risk *(38)*. Advantages and limitations of these methods have been previously described *(37–40)*.

[c]The results of the subsequent analyses did not differ when historical CD4 count nadir was used instead of CD4 count as a covariate in the ANOVAs.

Table 2
Measures of Immune Status, HIV Symptom Classification Prevalence, and HAART Regimen Prevalence and Duration for the HIV+ Groups With and Without Protease Inhibitor Exposure

Measure[a,b]	HIV+PI+	HIV+PI−	*p* value
CD4 (cells/μL)	362.0 ± 42.3	585.5 ± 43.8	[c]PI+ < PI−
Historical CD4 nadir (cells/μL)	142.0 ± 21.6	290.1 ± 38.5	[d]PI+ < PI−
CD8 (cells/μL)	992.7 ± 75.1	942.4 ± 60.8	—
B (cells/μL)	279.0 ± 27.1	280.5 ± 22.1	—
NK (cells/μL)	101.1 ± 12.4	95.7 ± 12.0	—
HIV-1 viral load (log)	2.8 ± 0.2	2.3 ± 0.1	[e]PI+ > PI−
HIV classification prevalence (%)			
HIV asymptomatic	17.1	26.8	—
HIV symptomatic	4.9	9.8	—
AIDS	78.1	63.4	—
HAART treatment duration (mo)	35.7 ± 3.5	34.5 ± 3.1	—
HAART regimen prevalence (%)			
NNRTI	0.0	2.4	—
NRTI/NtRTI	80.5	39.0	[f]PI+ > PI−
NNRTI + NRTI/NtRTI	19.5	58.5	[g]PI+ < PI−

[a]Values are mean ± SE unless otherwise indicated.

[b]HIV+PI+, HIV-infected protease-inhibitor-exposure group; HIV+PI−, HIV-infected protease inhibitor nonexposure group; CD4, helper T cell; CD8, cytotoxic/suppressor T cell; B, B cell; NK, natural killer cell; HIV, human immunodeficiency virus; AIDS, acquired immune deficiency syndrome; HAART, highly active antiretroviral therapy; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NtRTI, nucleotide reverse transcriptase inhibitor.

[c]$F(1, 80) = 13.5$, $p < 0.001$.

[d]$F(1, 70) = 11.6$, $p < 0.002$.

[e]$F(1, 80) = 3.3$, $p < 0.08$.

[f]$Z = 2.43$, $p < 0.03$.

[g]$Z = 2.83$, $p < 0.008$.

for the three groups. No significant group differences were observed for systolic blood pressure, HDL-C, or M. However, there were significant differences between HIV+ and HIV− groups for total cholesterol (TC), TC/HDL-C ratio, LDL, WHR, triglycerides, CRP, and 8-isoprostane. All of these results remained significant when total family income, smoking prevalence, and cannabis prevalence were controlled; in addition, HIV group differences on diastolic blood pressure became significant following covariate analysis. Analyses examining differences between the HIV+PI+ and HIV+PI− groups revealed no significant differences, except for triglyceride levels, which remained significant when controlling for diabetes status, CD4 count, and HIV viral load. Notably, *post hoc* analyses of triglyceride and 8-isoprostane values comparing the HIV+PI− and HIV− groups revealed no significant difference, indicating that the HIV serostatus differences on these measures were a result of greater values in the HIV+PI+ group than in the other groups.

The proportions of subjects per HIV serostatus group whose MSX levels exceeded clinical standards for CHD risk classification were examined (cf. 37). A significantly greater proportion of HIV+ compared with HIV− control subjects, respectively, evidenced clinically elevated levels of TC/HDL ratio (39.0% vs 19.5%; $\chi^2(1) = 7.5$, $p < 0.007$) and LDL-C (42.7% vs 23.2%; $\chi^2(1) = 7.1$, $p < 0.008$); no difference in group proportions for clinically abnormal levels of total cholesterol (39% vs 31.7%), triglycerides (31.7% vs 20.7%), waist girth (29.3% vs 23.2%), and insulin resistance (47.6% vs 39%) was observed. A significantly greater proportion of HIV+PI+ compared with HIV+PI− subjects, respectively, evidenced elevated levels of TC/HDL ratio (51.2% vs 26.8%; $\chi^{2+}(1) = 5.1$, $p < 0.03$); no difference in HAART group proportions for abnormal levels of total cholesterol (41.5% vs 36.6%), LDL-C (46.3% vs 39.0%), triglycerides (39.0% vs 24.4%), waist girth (24.4% vs 34.2%), and insulin resistance (53.7% vs 41.5%) was observed. Therefore, as can be seen from Table 3,

Table 3
Mean ± SE Values for MSX, Inflammation, Oxidative Stress, and Other CHD Risk Indices for the HAART Groups, and the HIV⁻ Control Group

Measure[a]		HIV⁺PI⁺	HIV⁺PI⁻	HIV⁻ control	Group differences
SBP	(mmHg)	113.9 ± 1.5	114.4 ± 2.1	116.1 ± 1.2	—
DBP	(mmHg)	79.6 ± 1.4	78.8 ± 1.9	76.7 ± 0.9	[b]HIV⁺ > HIV⁻
M	(mg/kg × (min)	5.4 ± 0.7	5.3 ± 0.5	6.2 ± 0.4	—
WHR		0.90 ± 0.01	0.91 ± 0.01	0.87 ± 0.01	[c]HIV⁺ > HIV⁻
TC/HDL-C		5.0 ± 0.2	4.5 ± 0.3	4.0 ± 0.2	[d]HIV⁺ > HIV⁻
TC	(mg/dL)	202.5 ± 8.4	194.6 ± 5.0	176.5 ± 4.1	[e]HIV⁺ > HIV⁻
HDL-C	(mg/dL)	43.2 ± 2.3	46.5 ± 2.1	46.7 ± 1.4	—
LDL-C	(mg/dL)	128.0 ± 6.4	123.0 ± 4.9	108.0 ± 3.6	[f]HIV⁺ > HIV⁻
Triglycerides	(mg/dL)	203.2 ± 34.0	130.7 ± 14.7	116.7 ± 11.7	[g]HIV⁺ > HIV⁻; PI⁺ > PI⁻; PI⁺ > HIV⁻
CRP	(mg/L)	7.5 ± 1.9	7.8 ± 2.5	1.8 ± 0.3	[h]HIV⁺ > HIV⁻
8-isoprostane	(pg/dL)	17.1 ± 4.1	10.8 ± 2.6	8.1 ± 0.3	[i]HIV⁺ > HIV⁻; PI⁺ > HIV⁻

[a]HIV⁺PI⁺, HIV-infected protease-inhibitor-exposure group; HIV⁺PI⁻, HIV-infected protease inhibitor nonexposure group; HIV⁻ control, HIV-seronegative healthy control group; SBP, systolic blood pressure; DBP, diastolic blood pressure; M, insulin sensitivity; WHR, waist-girth to hip-girth ratio; TC/HDL-C, ratio of total cholesterol to high-density lipoprotein cholesterol; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein; 8-isoprostane, 8-epi-prostaglandin $F_{2\alpha}$.
[b]$t(158) = 2.1$, $p < 0.04$, after controlling for covariates.
[c]$t(161) = 13.9$, $p < 0.004$.
[d]$t(161) = 3.4$, $p < 0.002$.
[e]$t(161) = 3.5$, $p < 0.003$.
[f]$t(161) = 3.2$, $p < 0.002$.
[g]$t(161) = 2.3$, $p < 0.03$; $t(161) = 2.3$, $p < 0.03$; $t(161) = 3.1$, $p < 0.003$.
[h]$t(161) = 3.8$, $p < 0.001$.
[i]$t(161) = 2.4$, $p < 0.02$; $t(161) = 2.7$, $p < 0.009$.

regardless of HAART treatment, HIV-seropositive status was associated with increased evidence of MSX (i.e., central obesity and dyslipidemia) and inflammation. Of note, however, is that HAART regimens that included PI medications were associated with greater triglyceride level, cholesterol ratio, and oxidative stress.

CHD Risk

Table 4 displays the estimated risk of an acute coronary event occurring within 10 yr for the three groups derived using the PROCAM and Framingham predictive model equations. In addition to the proportions of subjects per group who are at high CHD risk are depicted. Analyses of PROCAM and Framingham scores and risk (%/year) indicated that HIV⁺ subjects had significantly greater coronary event probability than did HIV⁻ subjects. The proportion of the HIV⁺ group with high risk (≥3.0%/yr) exceeded that of the HIV⁻ group. In addition, *post hoc* contrasts indicated that significantly greater event probabilities were observed for HIV⁺PI⁺ subjects than the HIV⁻ subjects for each measure of risk; moreover, when using the PROCAM method, the risk for the HIV⁺PI⁺ subjects also exceeded that observed for the HIV⁺PI⁻ subjects . Specifically, PI exposure was associated, respectively, for PROCAM or Framingham methods with 56% or 27% greater risk than for those not treated with PIs and with 129% and 50% greater risk than for HIV⁻ subjects. In sum, both methods for assessing future myocardial infarction risk indicated that HIV⁺ subjects and especially those persons on HAART regimens containing PIs are at heightened CHD risk.

Discussion

The main findings of this study were that (1) HIV⁺ subjects displayed greater inflammation levels than did HIV⁻ subjects; (2) HIV⁺ subjects showed greater

Table 4
Mean ± SE Risk of an Acute Coronary Event Occurring Within 10 Yr for PI-Exposure Groups and HIV$^-$ Control Group Derived Using the PROCAM and Framingham Predictive Model Equations

Measure[a]	HIV$^+$PI$^+$	HIV$^+$PI$^-$	HIV- control	Group differences
PROCAM risk score	28.1 ± 1.9	24.2 ± 1.7	19.3 ± 1.1	[b]HIV$^+$ > HIV$^-$; PI$^+$ > HIV$^-$
PROCAM risk (%/yr)	3.9 ± 0.8	2.5 ± 0.4	1.7 ± 0.2	[c]HIV$^+$ > HIV$^-$; PI$^+$ > HIV$^-$; PI$^+$ > PI$^-$
Framingham risk score	1.7 ± 0.6	0.2 ± 0.7	−1.0 ± 0.5	[d]HIV$^+$ > HIV$^-$; PI$^+$ > HIV$^-$
Framingham risk (%/yr)	3.3 ± 0.4	2.6 ± 0.3	2.2 ± 0.1	[e]HIV$^+$ > HIV$^-$; PI$^+$ > HIV$^-$
PROCAM: Proportion (%) of subjects with risk ≥ 3%/yr	36.6	24.4	13.4	[f]HIV$^+$ > HIV$^-$
Framingham: Proportion (%) of subjects with risk ≥ 3%/yr	53.7	39.0	31.7	[g]HIV$^+$ > HIV$^-$

[a]HIV$^+$PI$^+$, HIV-infected protease-inhibitor-exposure group; HIV$^+$PI$^-$, HIV-infected protease inhibitor nonexposure group; HIV$^-$ control, HIV-seronegative healthy control group.
[b]$t(161) = 4.1$, $p < 0.001$; $t(161) = 4.2$, $p < 0.001$.
[c]$t(161) = 3.3$, $p < 0.002$; $t(161) = 3.9$, $p < 0.001$; $t(161) = 2.2$, $p < 0.04$.
[d]$t(161) = 2.9$, $p < 0.005$; $t(161) = 3.3$, $p < 0.002$.
[e]$t(161) = 2.8$, $p < 0.008$; $t(161) = 3.3$, $p < 0.002$.
[f]$\chi^2(1) = 7.0$, $p < 0.009$.
[g]$\chi^2(1) = 3.7$, $p = 0.055$.

evidence of MSX than did HIV$^-$ subjects; and (3) HIV$^+$ subjects on PIs displayed greater oxidative stress, triglyceridemia, lipidemia, and risk of an acute coronary event within 10 yr than did non-PI-exposed HIV$^+$ subjects and their seronegative counterparts. These findings support the growing body of literature that implicates HAART medication as a potential etiopathological source of the increased prevalence of premature CHD in HIV$^+$ persons. However, the findings also add fuel to the debate concerning whether the heightened CHD risk is more a consequence of HIV infection itself than the medications used to treat the disease.

With the extended longevity of HIV$^+$ patients since the advent of HAART, the effects of competing morbidity and mortality risks including cardiovascular complications have become more apparent *(41)* and may be related to factors linked with chronic HIV infection. The findings of this study indicate that, regardless of HAART regimen, HIV-seropositive status was associated with increased evidence of MSX. Specifically, even though there was no difference between the groups in body mass, HIV$^+$ subjects compared with their matched-control counterparts had greater abdominal girth, albeit still within the normative range; this tendency toward greater central fat deposition is a major predictor in preclinical and clinical groups of other MSX indices and hence reflects increased cardiovascular disease risk *(42–45)*. Compared with controls, the HIV$^+$ subjects displayed greater dyslipidemia as well. Relative to clinical standards of high CHD risk classification *(37)*, the present findings indicated about a twofold greater proportion of HIV$^+$ subjects than controls with abnormal levels of TC/HDL ratio and LDL-C. Thus, HIV seropositivity was associated with differences in central obesity and with clinically significant lipidemia.

Recent studies of HIV$^+$ persons have reported evidence of a metabolic derangement that includes decreased insulin sensitivity *(46–50)*. It should be noted that hepatic hyperlipogenesis, hypertriglyceridemia, and insulin resistance have been observed in HIV$^+$ children and adults prior to the initiation of HAART *(51–54)*. Although greater insulin resistance in HIV$^+$ than in HIV$^-$ subjects was apparent in this study (*see* Table 3), no significant differences were observed. Only a small fraction of the HIV$^+$ subjects were diabetic and although the majority of HIV$^+$ persons were AIDS classified, most were asymptomatic. The pre-

vious findings of greater insulin resistance in some but not other studies of HIV+ persons suggests the lack of significant difference herein may reflect the tendency for alterations in insulin metabolism to occur at more advanced stages of disease progression. In this study, HIV+ persons with HCV coinfection were excluded. To date, only one study has examined the role of HCV coinfection, although HAART medications were not controlled; greater insulin resistance was observed in HCV-coinfected persons than in HIV+ persons not coinfected with HCV *(55)*. Therefore, an alternative explanation for the lack of insulin resistance group difference in this study is that greater numbers of HCV-coinfected persons were present in the studies in which differences were observed.

One robust finding in this study was that PI exposure, independent of diabetes status and indices of HIV disease progression (i.e., CD4 count, HIV viral load), was associated with greater triglyceride levels than non-PI exposure. Further support that these differences were not a consequence of spurious influences was indicated by the fact that a number of demographic, anthropometric, immune, and traditional cardiovascular risk factors (i.e., sex, ethnicity, income, education, body mass, age, HCV serostatus, family history of cardiovascular disease, smoking, and substance use) were controlled or evidenced no differences between groups. These triglyceride differences are in agreement with other reports; although many of these studies also report differences in cholesterol levels as a function of PI therapy (cf. *56*). In this study, an almost twofold greater number of those treated with PIs compared with non-PI-exposed subjects displayed cholesterol ratio levels that were in the high-risk range according to clinical standards. Of note, however, is that with a larger sample, significant differences in TC/HDL-C ratio would be observed in those subjects in this study with HAART regimens containing PIs.[d] Nevertheless, the presence of a potential PI effect on triglyceridemia was more readily observed, which suggests that PIs may have a more primary effect on triglyceride metabolism.

Some researchers have suggested that the role of triglycerides in atherosclerosis has been underestimated *(57,58)*. The mechanisms by which PIs influence lipid and carbohydrate metabolisms remain to be confirmed. PIs have been reported to have about 60% homology to regions within two proteins (i.e., CRABP-1, LRP) that are involved in lipid metabolism and when bound result in decreased hepatic triglyceride and lipid clearance *(59)*. It should be noted that the proportion of medication types within the two HAART groups in this study differs; the PI-exposed group included fewer subjects taking NNRTI medications in their regimen than in the non-PI-exposed group. Therefore, caution should be exerted when attributing the present differences between HAART groups as being solely a result of PI exposure. As previously suggested, it is more likely that a multifactorial pathogenesis of disease-, therapy-, and patient-related factors rather than a single drug-related mechanism can account for the complex pattern of metabolic disturbances observed in HIV spectrum disease *(60)*.

Previously, there has been no demonstration within the HIV literature that proinflammatory and oxidative stress mechanisms are associated with CHD risk measures. In this study, HIV+ subjects compared with their matched seronegative counterparts evidenced greater inflammation and oxidative stress levels. Specifically, 325% and 72% greater inflammation and oxidative stress levels, respectively, were observed in the HIV+ than in the control subjects. The possibility in HIV+ subjects that these markedly elevated levels are facilitating MSX processes consequent to their interaction with aspects of HIV spectrum disease or its treatment requires further investigation. The fact that greater levels of oxidative stress and not of inflammation were observed as a function of HAART medication suggests that oxidative stress may be involved in the underlying mechanisms facilitating greater triglyceridemia and lipidemia in the PI-exposed group.

The literature indicates that chronic immunosuppression in HIV+ men and women results in increased viral replication and diminished infection surveillance of other pathogens, and consequently increased levels of proinflammatory cytokines *(61,62)*. In HIV spectrum disease, greater levels of proinflammatory cytokines also stimulate greater HIV replication *(63)*. The HIV virion is a powerful polyclonal activator

[d]ANOVA comparison of TC/HDL ratio values between HIV+PI+ and HIV+PI− groups, with CD4 and HIV viral load controlled, revealed a nonsignificant difference ($t(78) = 1.7$, $p = 0.10$). A power analysis ($\alpha = 0.05$, power = 0.80) indicated that this difference would be significant with 105 subjects per group.

and, in turn, stimulates high levels of proinflammatory cytokines. When LDL cholesterol particles become trapped in an artery, they can undergo progressive oxygenation and can be internalized by macrophage *(9,13)*. This process is the normal protective operation of the macrophage consequent to the inflammatory stimulus. However, in the presence of chronic proinflammatory conditions and intracellular accumulation of triglycerides, the enhanced formation of oxygen radical species (in part by hepatic Kupffer cells) facilitates the collection of cholesterol and results in the formation of foam cells that become activated by LDL *(64)*. The proinflammatory condition becomes enhanced because the oxidized LDL can injure the vessel cells, which release inflammatory cytokines in response, and also can initiate a series of intracellular events that result in inflammatory up-regulation *(65)*. In HIV, the T-cell activation in the presence of multiple chronic infectious sources may exacerbate these pathophysiological mechanisms *(66)*. Consequently, a vicious circle may exist in which proinflammatory factors and triglycerides drive oxygen radical formation that, in turn, induce vascular injury, increased viral replication, and immune activation, leading to further inflammation that is maintained in the artery by conditions of exposure to chronic infectious sources and circulatory factors (e.g., turbulent blood flow) and by the presence of lipids *(67–70)*. The methods for estimating risk of a future acute myocardial infarction indicated that the HIV^+ subjects in this study relative to controls and especially those seropositive subjects on HAART regimens containing PIs were at heightened CHD risk.[e] The data from this study suggest that an interaction between triglycerides and oxidative stress on the one hand and PI exposure on the other may exacerbate MSX and CHD risk in HIV-infected individuals. Further research is needed to explore the potential roles of these factors in the context of chronic infection burden and inflammation in the cardiovascular pathophysiology of HIV spectrum disease.

[e]Depending on whether risk was estimated with Framingham or PROCAM methods, PI exposure was associated, respectively, with 27–56% greater risk than for those not treated with PIs and 50–129% greater risk than for seronegative controls. These findings are reasonable given the present results, but the categorical methods by which these prediction models derive these estimates have not been validated in HIV-infected populations and may perhaps overestimate CHD risk.

Acknowledgments

This research was supported by one research grant DA13128 from the National Institute on Drug Abuse of the National Institutes of Health, and two research grants HL36588 and HL72712 from the National Heart, Lung, and Blood Institute of the National Institutes of Health. In addition, we gratefully recognize the technical contributions of the following people to this research: Melanie Ashby, BA; Carmen Baez-Garcia, BA; David Carvajal; Luis Garcia; Alex Gonzalez, BA; Manuel Gonzalez, MD; Nancy Gonzalez, RN; Blair Hall; Nicole Henry, BA; Jimmy Heredia; Saleh Hernandez-Cassis, MD; Luda Karnatovskaia, MS; Pedro Martin, MD; Joanna Moose, RN; Maggie Olsson-Istel; Anna Rusiewicz, MS; Adriana Silva; Giselle Thompson, RN; Thelma Vargas; John Vaughan, RN; and Paul Wachowiak, BS.

References

1 Vigouroux, C., Gharakhanian, S., Salhi, Y., Nguyen, T.H., Adda, N., Rozenbaum, W., et al. (1999). Adverse metabolic disorders during highly active antiretroviral treatments (HAART) of HIV disease. *Diabetes Metab.* **25**:383–392.

2 John, M., Nolan, D., and Mallal, S. (2001). Antiretroviral therapy and the lipodystrophy syndrome. *Antivir. Ther.* **6**:9–20.

3 Nolan, D. and Mallal, S. (2001). Getting to the HAART of insulin resistance. *AIDS* **15**:2037–2041.

4 Reaven, G.M. (1995). Pathophysiology of insulin resistance in human disease. *Physiol. Rev.* **75**:473–486.

5. Schneiderman, N. and Skyler, J.S. (1996). Insulin metabolism, sympathetic nervous system regulation, and coronary heart disease prevention, in *Behavioral Medicine Approaches to Cardiovascular Disease Prevention* (Orth-Gomer, K. and Schneiderman, N., eds.), Lawrence Erlbaum Associates, Mahwah, NJ, pp. 105–133.

6 Dube, M.P., Sprecher, D., Henry, W.K., Aberg, J.A., Torriani, F.J., Hodis, H.N., et al. (2000). Preliminary guidelines for the evaluation and management of dyslipidemia in adults infected with human immunodeficiency virus and receiving antiretroviral therapy: recommendations of the Adult AIDS Clinical Trial Group Cardiovascular Disease Focus Group. *Clin. Infect. Dis.* **31**:1216–1224.

7 Hadigan, C., Meigs, J.B., Corcoran, C., Rietschel, P., Piecuch, S., Basgoz, N., et al. (2001). Metabolic abnormalities and cardiovascular disease risk factors in adults with human immunodeficiency virus infection and lipodystrophy. *Clin. Infect. Dis.* **32**:130–139.

8 Mynarcik, D.C., McNurlan, M.A., Steigbigel, R.T., Fuhrer, J., and Gelato, M.C. (2000). Association of severe insulin resistance with both loss of limb fat and elevated serum tumor necrosis factor receptor levels in HIV lipodystrophy. *J. Acquir. Immune Defic. Syndr.* **25**:312–321.

9 Ross, R. (1999). Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* **340**:115–126.
10 Dinarello, C.A. (1989). Interleukin-1 and its biologically related cytokines. *Adv. Immunol.* **44**:153–205.
11 Aldhahi, W. and Hamdy, O. (2003). Adipokines, inflammation, and the endothelium in diabetes. *Curr. Diab. Rep.* **3**:293–298.
12 Birks, E.J. and Yacoub, M.H. (1997). The role of nitric oxide and cytokines in heart failure. *Coron. Artery Dis.* **8**:389–402.
13 Witztum, J.L. (1994). The oxidation hypothesis of atherosclerosis. *Lancet* **344**:793–798.
14 Barbaro, G. (2001). Cardiovascular manifestations of HIV infection. *J. R. Soc. Med.* **94**:384–390.
15 Libby, P. and Ridker, P.M. (1999). Novel inflammatory markers of coronary risk: theory versus practice. *Circulation* **100**:1148–1150.
16 Lagrand, W.K., Visser, C.A., Hermens, W.T., Niessen, H.W., Verheugt, F.W., Wolbink, G.J., et al. (1999). C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon? *Circulation* **100**:96–102.
17 Ridker, P.M., Hennekens, C.H., Buring, J.E., and Rifai, N. (2000). C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N. Engl. J. Med.* **342**:836–843.
18 Sklar, P. and Masur, H. (2003). HIV infection and cardiovascular disease—is there really a link? *N. Engl. J. Med.* **349**:2065–2067.
19 Mason, A.L., Lau, J.Y., Hoang, N., Qian, K., Alexander, G.J., Xu, L., et al. (1999). Association of diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* **29**: 328–333.
20 Ockenga, J., Tillmann, H.L., Trautwein, C., Stoll, M., Manns, M.P., and Schmidt, R.E. (1997). Hepatitis B and C in HIV-infected patients: prevalence and prognostic value. *J. Hepatol.* **27**:18–24.
21. Centers for Disease Control, CDC. (1993). 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *JAMA* **6**:729–730.
22. The Florida Division of Disease Control Quarterly Surveillance Report: Bureau of HIV/AIDS. March 2002, Number 211. http://www.doh.state.fl.us/disease_ctrl/aids/trends/msr/msr.html
23. Brownley, K.A., Milanovich, J.R., Motivala, S.J., Schneiderman, N., Fillion, L., Graves, J.A., et al. (2001). Autonomic and cardiovascular function in HIV spectrum disease: early indications of cardiac pathophysiology. *Clin. Auton. Res.* **11**:319–326.
24. DeFronzo, R.A., Tobin, J.D., and Andres, R. (1979). The glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am. J. Physiol.* **6**:E214–E223.
25. Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W., and Fu, P.C. (1974). Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20**:470–475.
26. McGowan, M.W., Artiss, J.D., Strandbergh, D.R., and Zak, B. (1983). A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin. Chem.* **29**:538–542.
27. Warnick, G.R., Benderson, J., and Albers, J.J. (1982). Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin. Chem.* **28**:1379–1388.
28. Friedewald, W.T., Levy, R.S., and Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. *Clin. Chem.* **18**:449–502.
29. Motivala, S.J., Hurwitz, B.E., Llabre, M.M., Klimas, N.G., Fletcher, M.A., Antoni, M.H., et al. (2003). Psychological distress is associated with decreased memory T-helper and B-cell counts in pre-AIDS HIV seropositive men and women but only in those with low viral load. *Psychosom. Med.* **65**:627–635.
30. Koning, A.J. and Silk, M.H. (1963). The 2-thiobarbituric acid reagent for determination of oxidative rancidity in fish oils. *J. Am. Oil Chem. Soc.* **40**:165–169.
31. Reilly, M.P., Delanty, N., Roy, L., Rokach, J., Callaghan, P.O., Crean, P., et al. (1997). Increased formation of the isoprostanes IPF2alpha-I and 8-epi-prostaglandin F2 alpha in acute coronary angioplasty: evidence for oxidant stress during coronary reperfusion in humans. *Circulation* **96**:3314–3320.
32. Pratico, D., Iuliano, L., Mauriello, A., Spagnoli, L., Lawson, J.A., Rokach, J., et al. (1997). Localization of distinct F2-isoprostanes in human atherosclerotic lesions. *J. Clin. Inves.* **100**:2028–2034.
33. Gopaul, N.K., Anggard, E.E., Mallet, A.I., Betteridge, D.J., Wolff, S.P., and Nourooz-Zadeh, J. (1995). Plasma 8-epi-PGF2 alpha levels are elevated in individuals with non-insulin dependent diabetes mellitus. *FEBS Lett.* **368**: 225–229.
34. Moore, K. and Roberts, L.J. (1998). Measurement of lipid peroxidation. *Free Radic. Res.* **28**:659–671.
35. Wilson, P.W., D'Agostino, R.B., Levy, D., Belanger, A.M., Silbershatz, H., and Kannel, W.B. (1998). Prediction of coronary heart disease using risk factor categories. *Circulation* **97**:1837–1847.
36. Assmann, G., Cullen, P., and Schulte, H. (2002). Simple scoring scheme for calculating the risk of acute coronary events based on the 10-year follow-up of the prospective cardiovascular Munster (PROCAM) study. *Circulation* **105**:310–315.
37. National Cholesterol Education Program (Adult Treatment Panel III). (2001). *JAMA* **285**:2486–2495.
38. Haq, I.U., Ramsay, L.E., Jackson, P.R., and Wallis, E.J. (1999). Prediction of coronary risk for primary prevention of coronary heart disease: a comparison of methods. *QJM* **92**:379–385.
39. Grundy, S.M., Pasternak, R., Greenland, P., Smith, S. Jr., and Fuster, V. (1999). AHA/ACC scientific statement: assessment of cardiovascular risk by use of multiple-risk-factor assessment equations: a statement for healthcare professionals from the American Heart Association and the American College of Cardiology. *J. Am. Coll. Cardiol.* **34**:1348–1359.
40. Hense, H.W., Schulte, H., Lowel, H., Assmann, G., and Keil, U. (2003). Framingham risk function overestimates risk of coronary heart disease in men and women from

Germany—results from the MONICA Augsburg and the PROCAM cohorts. *Eur. Heart J.* **24**:937–945.
41. Vittecoq, D., Escaut, L., Merad, M., Teicher, E., Monsuez, J.J., and Chironi, G. (2003). Coronary heart disease in HIV-infected individuals, in *Advances in Cardiology: HIV Infection and the Cardiovascular System* (Barbaro, G., ed.), Karger, New York, pp. 151–162.
42. Goldstein, R.Z., Hurwitz, B.E., Llabre, M.M., Schneiderman, N., Gutt, M., Skyler, J.S., et al. (2001). Modeling preclinical cardiovascular risk for use in epidemiological studies: the Miami Community Health Study. *Am. J. Epidemiol.* **154**:765–776.
43. Haffner, S.M., Fong, D., Hazuda, H.P., Pugh, J.A., and Patterson, J.K. (1988). Hyperinsulinemia, upper body adiposity, and cardiovascular risk factors in non-diabetics. *Metabolism* **37**:338–345.
44. Shen, B.J., Todaro, J.F., Niaura, R., McCaffery, J.M., Zhang, J., Spiro, A. III, et al. (2003). Are metabolic risk factors one unified syndrome? Modeling the structure of the metabolic syndrome X. *Am. J. Epidemiol.* **157**:701–711.
45. Niaura, R., Todaro, J.F., Stroud, L., Spiro, A. III, Ward, K.D., and Weiss, S. (2002). Hostility, the metabolic syndrome, and incident coronary heart disease. *Health Psychol.* **21**:588–593.
46. Carr, A., Samaras, K., Burton, S., Law, M., Freund, J., Chisholm, D.J., et al. (1998). A syndrome of peripheral lipodystrophy hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *AIDS* **12**:F37–F39.
47. Dube, M.P., Johnson, D.L., Currier, J.S., and Leedom, J.M. (1997). Protease inhibitor-associated hyperglycemia. *Lancet* **350**:713–714.
48. Meyer, L., Rabaud, C., Ziegler, O., May, T., and Drouin, P. (1998). Protease inhibitors, diabetes mellitus and blood lipids. *Diabetes Metab.* **24**:547–549.
49. Walli, R., Herfort, O., Michl, G.M., Demant, T., Jager, H., Dieterle, C., et al. (1998). Treatment with protease inhibitors associated with peripheral insulin resistance and impaired oral glucose tolerance in HIV-1 infected patients. *AIDS* **12**:F167–F173.
50. Martinez, E. and Gatell, J.M. (1999). Metabolic abnormalities and body fat redistribution in HIV-1 infected patients: the lipodystrophy syndrome. *Curr. Opin. Infect. Dis.* **12**:13–19.
51. Geffner, M.E., Yeh, D.Y., Landaw, E.M., Scott, M.L., Stiehm, E.R., Bryson, Y.J., et al. (1993). In vitro insulin-like growth factor-I, growth hormone, and insulin resistance occurs in symptomatic human immunodeficiency virus-1-infected children. *Pediatr. Res.* **34**:66–72.
52. Hellerstein, M.K., Grunfeld, C., Wu, K., Christiansen, M., Kaempfer, S., Kletke, C., et al. (1993). Increased de novo hepatic lipogenesis in human immunodeficiency virus infection. *J. Clin. Endocrinol. Metab.* **73**:559–565.
53. Grunwald, C., Kotler, D.P. Hamadeh, R., Tierney, A., Wang, J., and Pierson R.N. (1989). Hypertriglyceridemia in the acquired immunodeficiency syndrome. *Am. J. Med.* **86**:27–31.
54. Constans, J., Pellegrin, J.L., Peuchant, E., Dumon, M.F., Pellegrin, I., Sergeant, C., et al. (1994). Plasma lipids in HIV-infected patients: a prospective study in 95 patients. *Eur. J. Clin. Invest.* **24**:416–420.
55. Duong, M., Petit, J.M., Piroth, L., Grappin, M., Buisson, M., Chavanet, P., et al. (2001). Association between insulin resistance and hepatitis C virus chronic infection in HIV-hepatitis C virus-coinfected patients undergoing antiretroviral therapy. *J. Acquir. Immune Defic. Syndr.* **27**:245–250.
56. Hoffman, C. and Jaeger, H. (2001). Cardiology and AIDS-HAART and the consequences. *Ann. NY Acad. Sci.* **946**: 130–144.
57. Cullen, P. (2000). Evidence that triglycerides are an independent coronary heart disease risk factor. *Am. J. Cardiol.* **86**:943–949.
58. Assmann, G. (2001). Pro and con: high-density lipoprotein, triglycerides, and other lipid subfractions are the future of lipid management. *Am. J. Cardiol.* **87(Suppl.)**:2B–7B.
59. Carr, A., Samaras, K., Chisholm, D.J., and Cooper, D.A. (1998). Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. *Lancet* **351**:1881–1883.
60. Behrens, G.M.N., Stoll, M., and Schmidt, R.E. (2003). Pathogenesis of the HAART-associated metabolic syndrome, in *Advances in Cardiology: HIV Infection and the Cardiovascular System* (Barbaro, G., ed.), Karger, New York, pp. 83–96.
61. Wilkinson, J. and Gotch, F. (2001). Immune interventions. *Br. Med. Bull.* **58**:187–203.
62. Gandhi, R.T. and Walker, B.D. (2002). Immunologic control of HIV-1. *Annu. Rev. Med.* **53**:149–172.
63. Yamamoto, N. (1994). The role of cytokines in the acquired immunodeficiency syndrome. *Int. J. Clin. Lab. Res.* **25**: 29–34.
64. Heine, R.J. and Dekker, J.M. (2002). Beyond postprandial hyperglycaemia: metabolic factors associated with cardiovascular disease. *Diabetologia* **45**:461–475.
65. Hajjar, D.P. and Haberland, M.E. (1997). Lipoprotein trafficking in vascular cells. Molecular Trojan horses and cellular saboteurs. *J. Biol. Chem.* **272**:22975–22978.
66. Hansson, G.K., Jonasson, L., Seifert, P.S., and Stemme, S. (1989). Immune mechanisms in atherosclerosis. *Arteriosclerosis* **9**:567–578.
67. Pelchen-Matthews, A., Signoret, N., Klasse, P.J., Fraile-Ramos, A., and Marsh, M. (1999). Chemokine receptor trafficking and viral replication. *Immunol. Rev.* **168**:33–49.
68. Peterhans, E. (1997). Reactive oxygen species and nitric oxide in viral diseases. *Biol. Trace Elem. Res.* **56**:107–116.
69. Piette, J. and Legrand-Poels, S. (1994). HIV-1 reactivation after an oxidative stress mediated by different reactive oxygen species. *Chem. Biol. Interact.* **91**:79–89.
70. Schreck, R., Rieber, P., and Baeuerle, P.A. (1991). Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *EMBO J.* **10**:2247–2258.