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Visceral adiposity, C-peptide levels, and low lipase activities predict HIV-dyslipidemia.

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Abstract. Protease inhibitor-based highly active antiretroviral therapy (PI-HAART) has been implicated in dyslipidemia, peripheral insulin resistance, and abnormal adipose tissue deposition in HIV/AIDS. *In vitro* evidence indicates that some PIs reduce adipocyte lipoprotein (LPL) and hepatic lipase (HL) expression and activities. We examined whether LPL and HL activities are reduced in HIV-infected patients with dyslipidemia. Fasting serum lipids, glucoregulatory hormones, post-heparin LPL and HL activities, as well as whole-body and regional adiposity were measured in: 19 HIV-seronegative controls, 9 HIV+ patients naïve to all anti-HIV medications, 9 HIV+ patients naïve to PI's, 9 HIV+ patients with prior PI experience but not currently receiving PIs, and 47 HIV+ patients receiving PI-HAART. The PI-HAART group had low LPL and HL activities. However, multiple linear regression analysis indicated that low post-heparin LPL activity only partially contributed to HIV-dyslipidemia. Central adiposity and high C-peptide levels (an indicator of high insulin secretion) were stronger predictors of HIV-dyslipidemia. Low LPL and HL activities, by themselves, were insufficient to explain HIV-dyslipidemia because the PI-naïve group had low LPL and HL activities, but had normal adiposity, C-peptide levels and serum lipid and lipoprotein levels. HDL-cholesterol was lower in PI-HAART and PI naïve groups than seronegative controls, and directly associated with LPL activity. These findings suggest that HIV-dyslipidemia is primarily mediated by factors that influence triglyceride and lipoprotein synthesis (eg., central adiposity and hyperinsulinemia), and only partially mediated by factors that influence triglyceride clearance (eg., lipase activity).

Key words: AIDS, metabolic complications, insulin resistance, central obesity, aspartyl protease inhibitors, magnetic resonance imaging.

Abbreviations: NRTI= nucleoside reverse transcriptase inhibitor, NNRTI= non-nucleoside reverse transcriptase inhibitor, PI= protease inhibitor, HAART= highly active antiretroviral therapy, AZT= zidovudine, 3TC= lamivudine, d4T= stavudine, ddI= didanosine, ddC= zalcitabine, EFV= efavirenz, NVP= nevirapine, DLV= delavirdine, IDV= indinavir, RTV= ritonavir, SQV= saquinavir, NFV= nelfinavir, APV= amprenavir, LPL= lipoprotein lipase, HL= hepatic lipase, $\text{INF}\alpha$ =interferon- α , MRI= magnetic resonance imaging, BMI= body mass index, SAT= abdomen subcutaneous adipose tissue, VAT= visceral adipose tissue, TAT= total abdominal adipose tissue, T:A= trunk:appendicular adipose tissue ratio.

Introduction. Highly active antiretroviral therapy (HAART) has dramatically reduced the morbidity and mortality rates associated with human immunodeficiency virus-1 infection (HIV (4)). However, HAART has been associated with several metabolic anomalies (28). HAART typically consists of 2 nucleoside-analog reverse transcriptase inhibitors (NRTI), combined with 1-2 inhibitors of the HIV-aspartyl protease (PI) or a non-nucleoside analog-reverse transcriptase inhibitor (NNRTI). Prior to the availability of PIs, NRTI ± NNRTI therapies were associated with mild hypertriglyceridemia that was attributed to an increased rate of hepatic lipogenesis (10, 12, 13, 15). Although very effective against HIV, the addition of PI to this regimen (PI-HAART) has been associated with severe hypertriglyceridemia (> 400mg/dL, > 4.5mM) (16). An increased rate of hepatic lipogenesis may still contribute to hypertriglyceridemia in the PI-HAART era, but reduced triglyceride clearance and conversion need to be examined.

Lipoprotein lipase (LPL) is the enzyme primarily responsible for triglyceride clearance from the circulation (7). LPL is synthesized and secreted from parenchymal tissues, transported to the capillary endothelium in skeletal muscle, adipose tissue, and heart, where it hydrolyzes lipoprotein triglycerides (chylomicrons, VLDL) to provide fatty acids to these tissues. Heparin administration releases LPL from the capillary endothelium, and post-heparin LPL activity is absent in type I hyperlipoproteinemia, a rare disorder associated with severe chylomicronemia and low to absent HDL-cholesterol (3). Hepatic lipase (HL) is an enzyme with considerable homology to LPL that is also capable of hydrolyzing triglycerides of plasma lipoproteins. HL is bound to the hepatocyte surface and like LPL, can be released into the circulation by heparin. Unlike LPL, the consequences of HL deficiency are poorly defined. In LPL deficiency, HDL levels are decreased while HL deficiency tends to be associated with increased HDL (6).

In vitro evidence from C3H10T1/2 murine mesenchymal stem cells (19), human and 3T3-L1 preadipocytes (32, 33), and 3T3-F442A adipocytes (26) indicates that incubation with varying amounts of several HIV-PIs inhibit adipocyte differentiation, LPL mRNA expression and LPL activity. In human embryonic kidney and hepatoma cell lines transfected with a LPL promoter construct that contained 3 putative sterol-regulatory elements, indinavir (IDV; one PI) reduced sterol regulatory element binding

protein-1c (SREBP-1c)-dependent LPL reporter gene activity. Notwithstanding the technical difficulties inherent with *in vitro* treatment of cells with HIV-PIs (18), these studies would predict that HIV-infected patients receiving PI-HAART would have low LPL activity, and this might contribute to HIV-associated dyslipidemia. One preliminary report found lower than normal post-heparin LPL and HL activities in 12 HIV-infected patients with severe hypertriglyceridemia (1). To test the hypothesis that impaired post-heparin LPL activity contributes to the dyslipidemia associated with PI-HAART, we measured post-heparin LPL and HL enzyme activities and lipid profiles in a cross-section of people living with HIV and treated with different antiviral regimens.

Materials and Methods.

Experimental Subjects. Seventy-four HIV-infected subjects and 19 seronegative controls were enrolled in this cross-sectional study. HIV-infected subjects were consecutively enrolled from the AIDS Clinical Trials Unit, the Infectious Disease Clinic at Washington University Medical School, and from several community practices. Most all subjects seen were included unless they: (A) were not fasting, (B) would/could not provide informed consent for the research procedures, (C) were habitual illegal drug users or known alcoholics, (D) were taking other medications that may affect glucose/lipid metabolism (ACE inhibitors, cyclosporine, furosemide, hydrochlorothiazides, lithium, prednisone, β -blockers, terbutaline, megestrol acetate, anabolic steroids, lipid or cholesterol-lowering agents), or (E) had a contraindication for heparin administration (history of pancreatitis or a blood clotting disorder). HIV-infected subjects were questioned about their antiviral medication history. For the majority of subjects, medical and pharmacy records were reviewed to document start and stop dates for all medications. We tabulated the number of weeks each patient received each medication, and calculated the average weeks of exposure to each medication (Figure 1).

Patients were assigned to a group on the basis of their current (at least the prior 6 months) medications. Groups were: HIV+ taking PI-based HAART (n=47; Table 1), HIV+ with PI experience, but no PI use within the prior 6 months (n=9), HIV+ but naïve to PI's (n=9), HIV+ with no exposure to anti-HIV medications (n=9), and seronegative controls (n=19). Subjects were queried about personal and family history (parents and siblings) of diabetes, cardiovascular disease (hypertension, myocardial infarct, bypass surgery, stroke) and hypercholesterolemia (>5.2mM), as well as exercise habits (> or <3 days/wk) and personal use of tobacco and alcohol (>3 drinks/day; Table 2). The Human Studies Committee at Washington University Medical School approved all procedures and informed consent was obtained from each volunteer prior to enrollment.

Body Composition. All subjects had height and weight measured while they wore minimal clothing or a hospital gown. Adipose and lean tissue mass in the trunk and appendicular regions was determined using a Hologic QDR 2000 dual-energy x-ray absorptiometer (DEXA) in 90% of the subjects.

A Hologic-certified radiology technician identified the regions of interest (arms, legs, trunk) and used Hologic Enhanced Array Whole Body software (v5.71A) to quantify bone mineral density, adipose and lean tissue mass in each region. Trunk: appendicular adipose tissue ratio (T:A) was calculated as trunk fat/(right and left arm and leg fat) as previously described (14).

Adipose tissue cross-sectional area in the abdomen was measured using ^1H -magnetic resonance imaging (MRI) in a subset of patients (77% of all subjects). A series of 3-5 axial MRI images of the abdomen (8 mm thick) were obtained at the level of lumbar 4-5 inter-vertebral space. Subcutaneous (SAT) and intra-abdominal (VAT) adipose tissue cross-sectional areas were measured in each axial image using NIH Image software (v1.61b7). The areas from serial images of the abdomen were averaged. The total abdominal adipose area was calculated (SAT+VAT=TAT) and intra-abdominal adiposity was expressed as VAT:TAT (21).

Serum Lipids and Lipoproteins. Blood was collected from an antecubital vein after an overnight (10-15 hr) fast. Serum triglycerides, total- and HDL-cholesterol were measured in the Core Laboratory for Clinical Studies at Washington University Medical Center. Serum total cholesterol and glycerol-blanked triglyceride concentrations were measured using enzymatic kits from Bayer and Diagnostics on a Hitachi 917 analyzer. HDL cholesterol concentration was measured as above after precipitating apo B-containing lipoproteins from serum using dextran sulfate (31). In samples with triglycerides ≤ 4.5 mM (<400 mg/dL), LDL cholesterol concentration was estimated using the Friedewald equation (8). In samples with triglycerides > 4.5 mM, LDL-cholesterol concentration was not measured. The accuracy of these methods is verified and standardized by participation in the CDC Lipid Standardization Program, the CDC Cholesterol Reference Method Laboratory Network, and the College of American Pathologists external proficiency program (23). Fasting insulin concentration was determined by a commercial laboratory (Linco Research Laboratories, Inc. St. Charles, MO) using a double-antibody radioimmunoassay (RIA). Fasting C-peptide and glucagon concentrations were determined by RIA.

Heparin-Releasable LPL and HL Activities. In all subjects, 60 units of heparin/kg were administered intravenously after the baseline blood collection. A blood sample was collected 10 min after heparin administration. Post-heparin plasma was incubated with high- and low-salt extraction buffers. LPL, but not HL activity, is inhibited by high-salt concentrations. The low-salt extract contains both LPL and HL activities and the high-salt fraction contains only HL activity. The difference between the lipase activities in low and high-salt fractions is equivalent to the LPL activity. LPL and HL activities were quantified *ex vivo* by measuring the rate at which post-heparin plasma converted ^{14}C -triolein to ^{14}C -free fatty acids under standardized conditions (60 min incubation at 37°C). ^{14}C -free fatty acids were extracted from the incubation and quantitated using a liquid scintillation counter (30). In separate experiments involving serial dilutions of post-heparin plasma, we determined that extremely high serum triglyceride levels did not artifactually reduce LPL activity in this *ex vivo* assay.

Statistics. Data are reported as mean \pm SEM. Differences among the groups were identified using ANOVA and the LSD post-hoc test where indicated. Pearson correlation analysis was used to identify linear associations between two variables. Multiple linear regression analysis was used to identify the best predictor(s) of hypertriglyceridemia after controlling for each of the variables that were related to triglyceride levels in this cohort. Predictors included in the multiple regression were C-peptide levels as an indicator of insulin secretion, DEXA-derived T:A ratio as an indicator of visceral adiposity, VAT:TAT, medication exposure, demographics, and lipase activities. The assumptions underlying the multiple linear regression model were examined. The predictors selected were linearly related to triglyceride levels. The residuals for each predictor were normally distributed and any individual outliers that excessively biased the model were removed. Predictors that contributed a high degree of multicollinearity were removed from the model (eg., insulin, VAT:TAT). Chi-square analysis was used to determine if a history of diabetes, cardiovascular disease, hypercholesterolemia, exercise, tobacco or alcohol use was independent of the group assignment. *P-values* <0.05 were considered statistically significant.

Results. All groups were similar with respect to age, gender and ethnic distribution (Table 1). Based on self-report, the patients who were naïve to all antiviral medications had been infected with HIV for a shorter time ($P < 0.03$). The group naïve to all HIV medications also had the highest viral load, but their CD4 count was similar to all HIV-infected groups. A personal or family history of diabetes, and a personal history of CV disease, ethanol use and regular exercise were independent of the group into which subjects were classified (Table 2). However, the proportion of subjects who reported a family history of CV disease, or a personal history of hypercholesterolemia or tobacco use was not equivalent among the 5 groups (Table 2).

Average exposure to all anti-HIV medications was not different among PI-HAART, prior PI experience, and PI-naïve groups (Figure 1). In the groups with PI experience, PI use was predominantly distributed among IDV, NFV, RTV, and SQV, while APV use was very low (Figure 1). When medication exposure was normalized to the duration of HIV infection there was no difference in exposure among the groups.

On average, all groups had similar body weight, BMI, and lean body mass, and percentage of body weight that was adipose tissue (Table 1). Despite this, trunk: appendicular (T:A) adipose ratio was greater in the PI-HAART group than in seronegative controls, subjects naïve to all medications, and subjects with prior PI experience ($p < 0.003$; Table 1). The PI-naïve group had a higher T:A ratio than the seronegative controls and the group that was naïve to all anti-HIV medications ($P < 0.041$). On the basis of the limited analysis of abdominal adipose tissue cross-sectional areas, the VAT: TAT ratio was greater in the PI-HAART group than in seronegative controls, subjects naïve to all medications, and subjects with prior PI experience ($p < 0.05$). Only the PI-HAART group had an average VAT: TAT > 0.40 ; a threshold value used to identify abnormally high intra-abdominal adiposity (21). Fat distribution in the PI-HAART group was “mixed”; ~16% of the PI-HAART subjects had a normal body fat distribution/appearance (ie., no unusual phenotype), ~30% of the PI-HAART subjects had severe peripheral fat atrophy without excessive central adiposity, and ~54% had modest to severe peripheral fat atrophy and central adipose tissue accumulation as their predominant phenotype. DEXA-derived trunk: appendicular adipose ratio

correlated with VAT: TAT ($r= 0.62$, $p\leq 0.0001$) in the 70 subjects on whom both measures were available. As a result, trunk: appendicular adipose ratio values were used to represent visceral adiposity in subsequent multiple regression analyses.

There were no differences in fasting insulin and glucagon concentrations among the 5 groups (Table 1). C-peptide levels were higher in PI-HAART than in control and naïve to all medication groups (Table 1). The PI-HAART and PI-naïve groups had lower HDL concentrations than the seronegative group ($p<0.04$). Fasting triglycerides (7.4 ± 1.8 mM) were higher in the PI-HAART group than the seronegative controls. Total-cholesterol levels (7.1 ± 0.7 mM) were higher in the PI-HAART group than in the control and PI-naïve groups (Table 1). The triglyceride, total- and HDL-cholesterol levels in the PI-HAART group, and the triglyceride and HDL-cholesterol levels in the PI-naïve group represent a high risk for CV disease by NCEP guidelines (23). Calculated LDL-cholesterol levels were not higher in the PI-HAART group, but the LDL level could not be calculated in 18 of the PI-HAART subjects because their triglyceride levels were > 4.5 mM.

Heparin releasable-LPL activity was lower in the PI-HAART group than in control, PI-naïve and past PI experience groups ($p<0.03$; Table 1). LPL activity was lower in the naïve to all HIV medications group than in the past PI experience group, but triglyceride and lipoprotein levels were normal in these 2 groups. HL activity was lower in PI-HAART than in PI-naïve, prior PI experience and the seronegative groups ($p<0.04$).

Heparin-releasable LPL and HL activities were inversely related to fasting serum triglycerides ($r=-0.305$; $P=0.003$ for LPL, $r=-0.215$; $P=0.04$; $n=93$ for HL; Figure 2). When the 7 subjects with the highest triglycerides were removed from the regression analysis, heparin-releasable LPL and HL activities remained inversely related to fasting serum triglycerides ($r=-0.258$; $P=0.02$ for LPL, $r=-0.261$; $P=0.02$ for HL; $n=86$; inset-Figure 2). When only the PI-HAART subjects were considered, LPL activity ($r=-0.404$; $P=0.005$; $n=47$), but not HL activity ($r=-0.258$; $P=0.080$; $n=47$) was inversely related to

serum triglycerides. LPL activity was directly associated with fasting HDL-cholesterol concentrations ($r=0.55$; $P \leq 0.0001$; $n=93$).

When all subjects were included in a multiple linear regression analysis where triglyceride level was the dependent variable, T:A ratio ($P=0.015$), C-peptide levels ($P=0.002$), and LPL activity ($P=0.030$) were significantly correlated (partial) with triglyceride levels. The model explained 40% of the variance ($P < 0.0001$). The predictive strength of this model was good; ie., the standard error of the estimate (± 244) was less than the standard deviation of the mean for triglyceride levels (± 315). Based on the standardized β coefficients, the relative importance of the 3 variables that significantly contributed to the model-predicted triglyceride level after controlling for each variable independently was highest for C-peptide levels (0.36), intermediate for T:A ratio (0.27), and lowest for LPL activity (-0.21).

Discussion. These findings suggest that low post-heparin LPL and HL activities only partially contributed to HIV-associated dyslipidemia. The presence of central adiposity and high C-peptide levels (an indication of high insulin secretion) were stronger predictors of HIV-dyslipidemia than low LPL and HL activities. Low LPL and HL activities, by themselves, were insufficient to explain HIV-dyslipidemia because we identified a group of HIV-infected subjects who were naïve to all antiviral medications, had normal adiposity, C-peptide levels and serum lipid and lipoprotein levels, but had low LPL and HL activities. As suggested previously (11-13, 15), perhaps a higher plasma HIV viremia reduced LPL and HL activities in these subjects. In conjunction with prior evidence, these findings suggest that several factors, including central adiposity, high insulin secretion, HIV-induced elevations in proinflammatory cytokines (12), accelerated rates of hepatic lipogenesis (15) and lipoprotein synthesis (20, 27), as well as reduced rates of lipoprotein clearance (29), contribute to HIV-associated dyslipidemia. On the basis of the present findings, we suggest that elevated rates of triglyceride and lipoprotein synthesis, perhaps induced by central adiposity and high insulin levels, contribute more to HIV-dyslipidemia than low rates of lipase-mediated triglyceride clearance.

Our findings are limited by the fact that we cannot determine the temporal relationships among the strongest predictors of HIV-dyslipidemia. For example, it is possible that PI-HAART directly reduced insulin sensitivity (17), indirectly increased visceral adiposity (9), and both directly inhibited LPL activity. Recent publications support the notion that trunk adiposity, hyperinsulinemia, and hypertriglyceridemia can be dissociated in people living with HIV and receiving PI-HAART (22, 24). Collectively, these findings support the hypothesis that HIV-related metabolic complications represent a combination of different syndromes, with different etiologies.

Other evidence from the present study supports the notion that the pathogenesis of HIV-dyslipidemia is multifactorial. The subjects naïve to all anti-HIV medications had low LPL and HL activities but normal serum triglyceride and cholesterol levels. Although this group had a higher viral load than the other groups studied, they did not have an AIDS-diagnosis. Perhaps having a slightly higher plasma viral load (1 log) reduced their lipase activities, but the level of plasma HIV viremia was not

sufficient to increase the rate of triglyceride synthesis (or triglyceride-rich lipoproteins), so their serum lipid levels were normal. The PI-naïve group had normal LPL and HL activities, but reduced HDL-cholesterol and a tendency toward elevated serum triglyceride levels. Their visceral adiposity (T:A ratio =1.33) probably contributed to their moderate dyslipidemia, perhaps through an accelerated rate of triglyceride synthesis. The PI-HAART group had visceral adiposity, high insulin and C-peptide levels, and low LPL and HL activities, but we cannot eliminate their history of tobacco use or genetic factors as contributors to HIV-dyslipidemia.

In support of the findings of Purnell et al. (25), post-heparin HL activity was lower in the PI-HAART group. Purnell et al. (25) administered RTV to non-obese, seronegative, normolipidemic subjects for 2 wk and found modest elevations in plasma triglycerides, no effect on post-heparin LPL activity, a reduction in HL activity, but HDL levels were not increased. The PI-HAART group in the current study had greater visceral adiposity, and low HL activity. Decreased HL activity has been associated with fewer, small, dense LDL particles, and higher HDL-cholesterol levels; both reflecting a more favorable lipid profile. We observed low HDL-cholesterol levels in the setting of low HL activity. LDL and HDL particle size/density were not measured in this study, so we cannot confirm the physiologic action of the low HL activity. However, PI-HAART subjects had significantly lower levels of HDL-cholesterol. LPL activity was directly related to HDL-cholesterol levels and HL activity did not significantly contribute to the multiple regression model that predicted triglyceride levels. This suggests that in this wide spectrum of HIV-infected subjects (from medication naïve to PI-HAART), low HL activity is not a primary mediator of HIV-dyslipidemia. LPL is known to be required for the generation of HDL-cholesterol (5), and physiologically induced changes in LPL activity directly affect HDL-cholesterol levels (30).

Purnell et al. did not find reduced post-heparin LPL activity in HIV-seronegative controls given RTV for 2 wks (25), while we found LPL activity was lower in PI-HAART recipients. Potential reasons for these different observations include: PI-HAART treated patients received antecedent treatment with NRTI or NNRTI medications followed by the addition of PIs, HIV-infected subjects may experience

multi-drug interactions that result in higher circulating drug concentrations (esp. with PIs), and our HIV-infected cohort may have had greater whole-body or visceral adipose deposition or greater baseline triglyceride or insulin levels. Purnell et al. (25) did not report adiposity or fasting insulin levels in their seronegative subjects.

These *in vivo* findings only modestly support previous *in vitro* studies (19, 26, 32, 33) that found some PIs or NRTIs have direct inhibitory effects on lipase expression, processing, secretion or activity, and might therefore contribute to PI-HAART-associated dyslipidemia. On the basis of the current findings, it seems unlikely that HIV-PIs have a potent inhibitory effect on proteases (known or unknown) that are involved in LPL and HL processing.

Another limitation to this cross-sectional study is that we do not know the body fat distribution of the subjects prior to HIV-infection or prior to initiation of antiviral therapy. The exceptionally high serum triglycerides observed in some PI-HAART subjects (>11.3 mM, >1000 mg/dL) may imply that these subjects have a different metabolic syndrome than subjects with lower triglycerides. Perhaps a certain genotype (eg., LPL polymorphism), lifestyle or behavioral factor made them more susceptible to dyslipidemia. As a result, antecedent adiposity, regional fat distribution, or insulin resistance may have contributed to dyslipidemia or reduced lipase activities.

One potential confounder is the use of the trunk: appendicular (T: A) adipose tissue ratio in subjects receiving PI-HAART. As noted by Safrin and Grunfeld (28), this ratio may be artifactually elevated in subjects with 2 different syndromes (ie., large trunk adipose depot vs. peripheral lipoatrophy). Both anthropomorphic alterations have been reported in PI-HAART, probably with different etiologies. In the current study, T: A adipose ratio measured using DEXA and VAT: TAT measured using MRI were correlated. This suggests that only minor errors are introduced when the DEXA-derived T: A adipose ratio is used to identify HIV-infected subjects with visceral adipose tissue deposition.

In summary, LPL activity was low in HIV-infected people treated with PIs, but visceral adiposity and high insulin secretion were more robust predictors of HIV-hypertriglyceridemia. An additional lipid abnormality was low HDL-cholesterol, a powerful determinant of cardiovascular risk (2), which implies

that PI-based regimens, while reducing morbidity and mortality, may predispose to cardiovascular disease. Given the risk associated with low HDL-cholesterol, it will be important to examine tissue-specific as well as fat depot-specific LPL expression and genetic polymorphisms for lipases in order to better define the mechanisms responsible for the metabolic complications of current therapies for people living with HIV.

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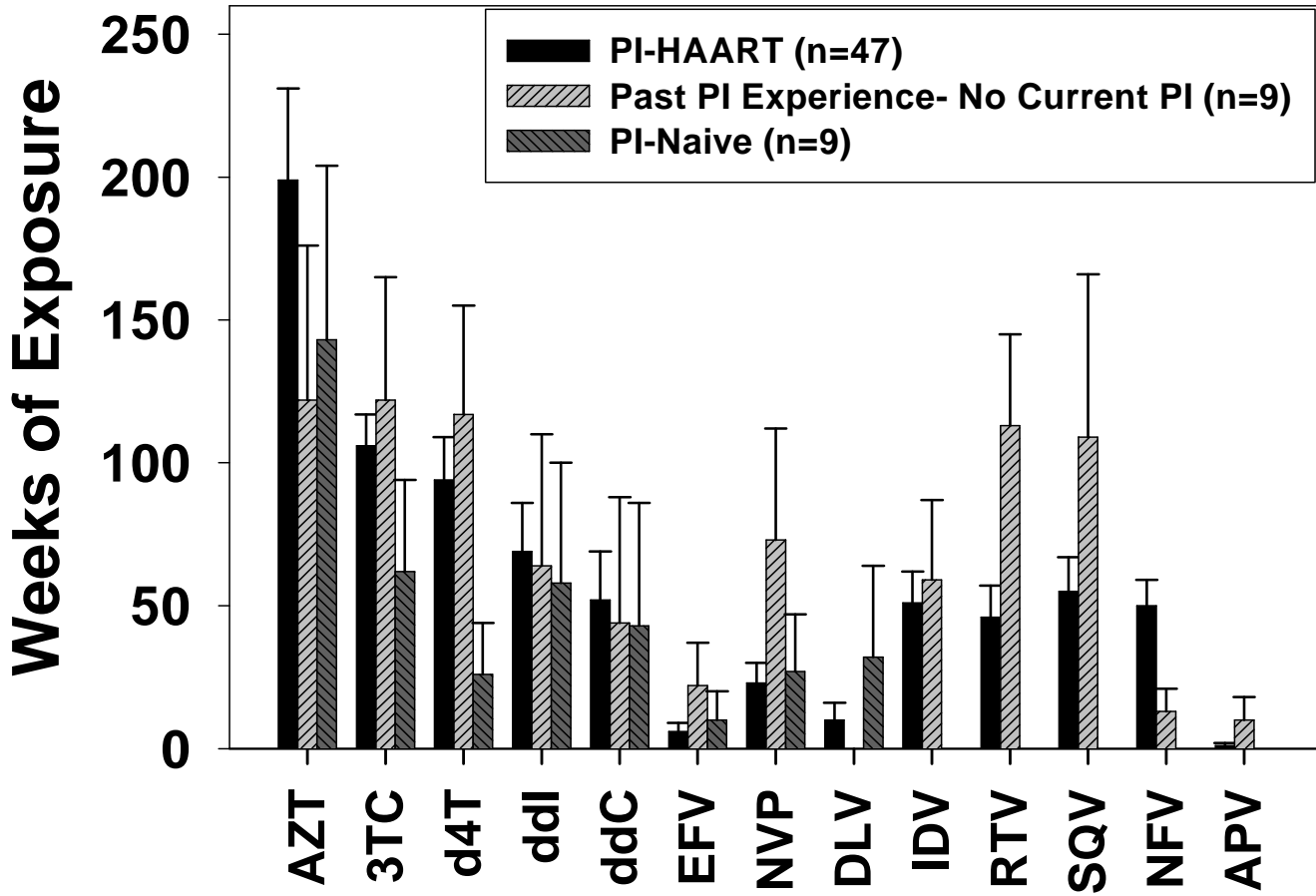
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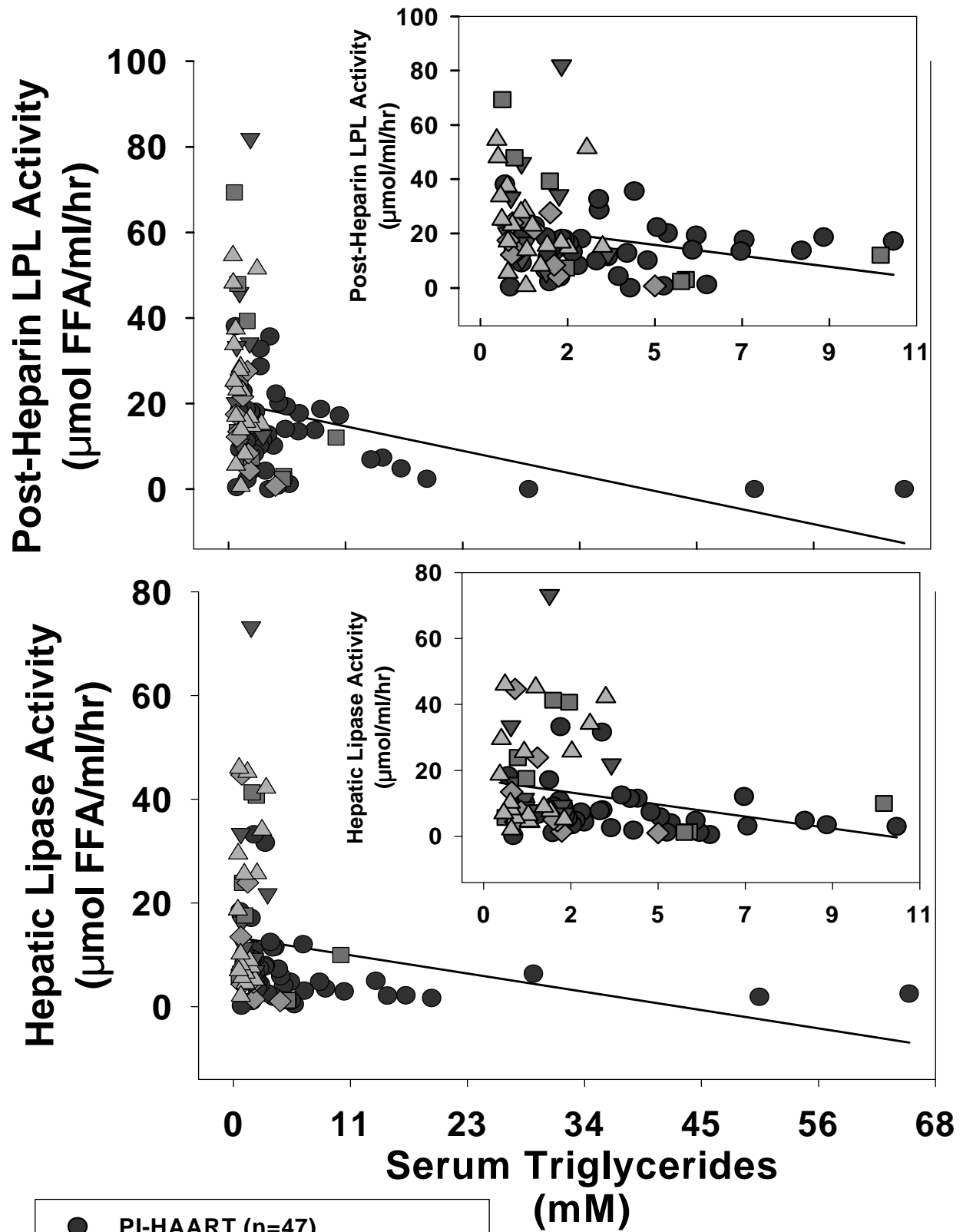
Figure Legends.

Figure 1. Medication exposure (weeks) was not different among the 3 HIV-infected groups receiving treatment. When the duration of medication exposure was normalized to the duration of HIV infection, there were no differences among the groups.

Figure 2. Fasting serum triglyceride concentrations were inversely related to heparin-releasable-LPL activity ($r = -0.305$; $P = 0.001$, $n = 93$) and HL activity ($r = -0.215$; $P = 0.02$; $n = 93$). The inset plots show the same associations after removing the 7 subjects with the highest triglycerides ($> 11\text{mM}$). Heparin-releasable LPL and HL activities remained inversely related to fasting triglycerides ($r = -0.258$; $P = 0.008$ for LPL, $r = -0.261$; $P = 0.008$ for HL; $n = 86$).

Figure 1.





- PI-HAART (n=47)
- ▼ Past PI Experience (n=9)
- PI-Naive (n=9)
- ◆ Naive To All Medications (n=9)
- ▲ Seronegative Controls (n=19)

Table 1. Subject Characteristics

PARAMETER	GROUP					P-value
	Seronegative	HIV+ Naïve to all Meds	HIV+ PI Naïve	HIV+ Prior PI Exper.	HIV+ PI-HAART	
	1	2	3	4	5	
n	19	9	9	9	47	
Age (yrs)	37 ± 2	36 ± 2	42 ± 3	36 ± 3	41 ± 1	ns
Men/Women	15/4	7/2	8/1	8/1	45/2	ns
C/AA/AP/H/O	16/0/1/1/1	7/2/0/0/0	8/1/0/0/0	3/6/0/0/0	40/2/0/5/0	ns
Yrs Known HIV+	na	1 ± 1	6 ± 2	6 ± 1	6 ± 1	<0.01 Grp 2 vs 3-5
HIV RNA (c/ml)	na	6 ± 3 × 10 ⁵	6 ± 4 × 10 ⁴	9 ± 4 × 10 ⁴	2 ± 1 × 10 ⁴	<0.002 Grp 2 vs 3-5
CD4 (cells/μL)	1014 ± 73	375 ± 107	341 ± 89	236 ± 69	455 ± 39	<0.0001 Grp 1 vs 2-5; <0.03 Grp 5 vs 4
Weight (kg)	80 ± 5	77 ± 6	81 ± 9	94 ± 7	82 ± 2	0.30
BMI (kg/m ²)	25 ± 1	27 ± 3	25 ± 4	31 ± 3	26 ± 1	0.21
Lean Body Mass (kg)	57 ± 3	52 ± 3	62 ± 4	61 ± 4	60 ± 1	0.10
% Body Fat	27 ± 2	29 ± 4	28 ± 4	31 ± 4	23 ± 1	0.11
T: A Adipose Ratio	0.90 ± 0.08	0.82 ± 0.07	1.33 ± 0.17	1.06 ± 0.13	1.60 ± 0.09	<0.003 Grp 5 vs 1,2 & 4 <0.041 Grp 3 vs 1 & 2
VAT/TAT	0.34 ± 0.03	0.31 ± 0.04	0.38 ± 0.05	0.35 ± 0.05	0.47 ± 0.03	<0.05 Grp 5 vs 1,2 & 4
Insulin (pM)	77 ± 8	74 ± 8	84 ± 15	137 ± 45	123 ± 16	0.21
C-peptide (nM)	0.49 ± 0.05	0.57 ± 0.09	0.71 ± 0.10	0.75 ± 0.14	0.86 ± 0.06	<0.032 Grp 5 vs 1 & 2
Glucagon (pM)	20 ± 2	23 ± 2	21 ± 2	32 ± 4	26 ± 2	0.06
Triglycerides (mM)	1.3 ± 0.2	1.7 ± 0.4	3.1 ± 1.1	1.5 ± 0.3	7.4 ± 1.8	<0.013 Grp 5 vs 1
Total Cholesterol (mM)	4.8 ± 0.2	4.5 ± 0.2	4.4 ± 0.4	4.7 ± 0.6	7.1 ± 0.7	<0.045 Grp 5 vs 1 & 3
HDL-Cholesterol (mM)	1.2 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	<0.037 Grp 5 vs 1,2 & 4 =0.02 Grp 1 vs 3
LDL-Cholesterol (mM)	3.0 ± 0.2	2.7 ± 0.3	2.1 ± 0.3	3.0 ± 0.5	3.2 ± 0.2	0.18
Post Heparin LPL Activity (μmol FFA/ml/hr)	24 ± 3	14 ± 3	24 ± 8	29 ± 8	13 ± 1	<0.03 Grp 5 vs 1,3 & 4; <0.03 Grp 2 vs 4
Hepatic Lipase Activity (μmol FFA/ml/hr)	18 ± 3	12 ± 5	16 ± 5	21 ± 7	7 ± 1	<0.04 Grp 5 vs 1,3 & 4

Table 2. Risk Factors/Family History

	GROUP					X ²
	Seronegative (n=19)	HIV+ Naïve to all Meds. (n=9)	HIV+ PI Naïve (n=9)	HIV+ Prior PI Exper. (n=9)	HIV+ PI-HAART (n=47)	
<u>(% Positive Response)</u>						
Pers. History Diabetes	0	0	11	11	6	2.76
Family History Diabetes	11	38	22	56	36	7.25
Pers. History CV Disease	0	0	0	11	2	4.18
Family History CV Disease	16	38	56	44	68	15.7*
Pers. History Hypercholesterolemia	5	0	11	0	28	9.63*
Pers. History Smoking	16	13	33	67	47	10.9*
Pers. History Ethanol	26	13	44	67	28	7.75
Regular Exercise	0	13	22	0	6	6.06

* $\alpha=0.05$ $df=4$ $X^2 > 9.49$