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Increased PAI-1 and tPA antigen levels are reduced with metformin therapy in HIV-infected patients with fat redistribution and insulin resistance

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Abstract Cardiovascular disease (CVD) risk associated with fat redistribution seen among HIV-infected individuals remains unknown, but may be increased due to hyperlipidemia, hyperinsulinemia, increased visceral adiposity, and a prothrombotic state associated with these metabolic abnormalities. In this study we characterized plasminogen activator inhibitor-1 (PAI-1) and tissue-type plasminogen activator (tPA) antigen levels, markers of fibrinolysis and increased CVD risk, in HIV lipodystrophic patients compared to controls. Furthermore, we investigated the effect of treatment with metformin on PAI-1 and tPA antigen levels in patients with HIV-associated fat redistribution. Eighty-six patients (age 43±1 yr, BMI 26.1±0.5 kg/m^2) with HIV and fat redistribution were compared to 258 age- and BMI-matched subjects from the Framingham Offspring study. In addition, 25 HIV-infected patients with fat redistribution and fasting insulin > 15 μU/mL [104 pmol/L] or impaired glucose tolerance, but without diabetes mellitus were enrolled in a placebo-controlled treatment study of metformin 500mg twice daily. PAI-1 and tPA antigen levels were significantly increased in patients with HIV related fat redistribution compared to Framingham control subjects (46.1±1.4 vs 18.9±0.9 μg/L PAI-1, 16.6±0.8 vs. 8.0±0.3 μg/L tPA, P=0.0001). Among patients with HIV infection, a multivariate regression analysis including age, sex, waist-to-hip ratio, BMI, smoking status, protease inhibitor use and insulin area under the curve (AUC), found gender and insulin AUC were significant predictors of tPA antigen. Twelve weeks of metformin treatment resulted in decreased tPA antigen levels (-1.9±1.4 vs +1.4±1.0 μg/L in the placebo-treated group P=0.02). Similarly, metformin resulted in improvement in PAI-1 levels (-8.7±2.3 vs +1.7±2.9 μg/L, P=0.03). Change in insulin AUC correlated significantly with change in tPA antigen (r=0.43, P=0.03). PAI-1 and tPA antigen, markers of impaired fibrinolysis and increased CVD risk, are increased in association with hyperinsulinemia in patients with HIV and fat redistribution. Metformin reduces PAI-1 and tPA antigen concentrations in these patients and may ultimately improve associated CVD risk.

Fat redistribution, dyslipidemia and insulin resistance have been recognized in patients with HIV infection(1-7). The cardiovascular risk associated with fat redistribution in these patients remains uncertain. Insulin resistance and impaired glucose tolerance have been shown to predict elevated cardiovascular disease risk in non-diabetic individuals without HIV infection (8-9) and to be associated with elevated concentrations of tissue type plasminogen activator (tPA) antigen and plasminogen activator inhibitor-1 (PAI-1)(10). High levels of PAI-1 and tPA antigen have been associated with increased risk of coronary artery disease. Increased tPA activity is indicative of increased fibrinolytic potential, however, tPA antigen assays measure both the small fraction of free tPA as well as inactive tPA complexed with PAI-1. Therefore, high levels of tPA antigen in the setting of high PAI-1 levels reflect impairment of the fibrinolytic system.

A prothrombotic state may exist in patients with HIV and fat redistribution, secondary to insulin resistance and increased visceral adiposity. Cardiovascular markers of impaired fibrinolysis have not been previously assessed in HIV infected patients with fat redistribution. The purpose of this study was: 1) to characterize tissue tPA antigen and PAI-1 levels in HIV-infected patients with fat redistribution compared to healthy control subjects, and 2) to determine the response of tPA and PAI-1 to metformin therapy in the treatment of insulin resistance associated with HIV-related lipodystrophy.

Methods

Cross-sectional Comparison with Framingham Control Subjects

Subjects: Eighty-six patients (62 men and 24 women) with HIV-infection reporting recent change in body fat distribution were prospectively evaluated between January and November 1999 at the Clinical Research Center of the Massachusetts Institute of Technology. Subjects were recruited using community-based advertisements seeking HIV-infected patients with fat-redistribution or were referred by their physicians for evaluation of fat redistribution. Subjects were screened by telephone and asked if they had experienced any of the following: 1) loss of fat in the face, 2) increased fat under the chin or back of the neck, 3) increased abdominal girth, 4) increased chest or breast fat, or 5) loss of fat in the arms or legs. Subjects who identified a change in fat distribution in any one or more body areas were invited to participate and fat redistribution was confirmed by physical examination in all subjects.

Subjects were excluded if they had changed antiviral medications within 6 weeks of the study, had a history of diabetes mellitus or previous treatment with an antidiabetic agent, reported use of testosterone, estrogen, growth hormone or other steroids in the past 6 months, had active alcohol or substance abuse or were not between 18-60 years of age. Written informed consent was obtained from each subject prior to testing in accordance with the Committee on Human Experimentation with Subjects of the Massachusetts Institute of Technology and the Subcommittee on Human Studies at the Massachusetts General Hospital. Body composition and metabolic data in a subset of these patients have been previously published (2).

Protocol: Each subject underwent a complete medical history and physical examination to confirm fat redistribution. Weight, height, body mass index (BMI), waist (at the level of the umbilicus), hip, mid-arm and mid-thigh circumference were determined (11). Following a 12 hour fast, a standard 75 gm oral glucose tolerance test was completed according to WHO standards (12) with determination of blood glucose and insulin levels at 0, 30, 60, 90 and 120 minutes, and insulin area under the curve (AUC). In addition, fasting blood samples were collected to determine PAI-1 and tPA antigen levels. Antiretroviral therapy was characterized as to current or past use and duration of protease inhibitor (PI), nucleoside reverse transcriptase inhibitor (NRTI), or nonnucleoside reverse transcriptase inhibitor (NNRTI) therapy.

Control Subjects: Control subjects were selected from the Framingham Offspring Study, a population-based observational study of risk factors for cardiovascular disease described previously (13-14). Controls were eligible for matching if they did not

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TABLE I. Clinical Characteristics of HIV Lipodystrophy Patients and Healthy Controls

	HIV Lipodystrophy (n=86)	Control (n=258)	p-value	adjusted p-value
Male/Female	62/24	186/72	—	—
Age (y)	43.1 (0.9)	43.7 (0.5)	0.6	—
BMI (kg/m^2)	26.1 (0.5)	26.2 (0.3)	0.9	—
Waist circumference (cm)	94.7 (1.2)	90.6 (0.8)	0.005	—
Hip circumference (cm)	97.7 (1.0)	100.5 (0.5)	0.01	—
WHR	0.97 (0.01)	0.90 (0.01)	0.0001	—
Duration HIV (y)	7.1 (0.4)	—	—	—
HIV Viral Load < 400 copies/ml †	65.3%	—	—	—
Current Smoker	30%	25%	0.35	—
Fasting Glucose (mg/dL) [mmol/L]	92.2 (1.4) [5.12 (0.08)]	93.5 (0.6) [5.19 (0.03)]	0.4	0.01
2 hr Glucose (mg/dL) [mmol/L]	136.3 (4.9) [7.56 (0.3)]	96.7 (1.5) [5.37 (0.08)]	0.0001	0.0001
Fasting Insulin (μU/mL) [pmol/L]	19.1 (1.8) [132.6 (12.5)]	7.9 (0.5) [54.9 (3.5)]	0.0001	0.0001
2 hr Insulin (μU/mL) [pmol/L]	101.0 (10.2) [701.4 (70.8)]	41.2 (2.5) [286.1 (17.4)]	0.0001	0.0001
Insulin AUC (μU/mL*120min) [pmol/L*120min]	10,367 (832) [71,999 (5778)]	N/A	—	—
PAI-1 (μg/L)	46.1 (1.4)	18.9 (0.9)	0.0001	0.0001
tPA (μg/L)	16.6 (0.8)	8.0 (0.3)	0.0001	0.0001

Values shown are mean(SEM). [SI units].
Adjusted p-values – represent multivariate linear regression p-value for group adjusting for WHR; † Viral load determination available on 72 patients with HIV infection

have diagnosed diabetes mellitus, were not using estrogen replacement therapy, and had complete information on all analytic covariates (n = 2959). We matched each case patient to 3 control subjects on sex, age (within 5 years) and BMI (within 1 kg/m^2). Data on control subjects were obtained between January 1991, and June 1995, during the fifth Offspring Study examination cycle. As with case patients, control subjects underwent a standardized medical history and physical examination, including measurement of height, weight, and waist (at the umbilicus), and hip circumference. After measurement of levels of fasting glucose, insulin, PAI-1 and tPA antigen, control subjects underwent a 75 gram oral glucose tolerance test administered according to WHO standards (12), and 120 minute post-challenge glucose and insulin levels were measured. Insulin AUC was not available from the Framingham control subjects.

Response to Metformin Therapy

Subjects: Thirty-three HIV infected subjects with fat redistribution were found to have impaired glucose tolerance (2 hour blood glucose between 140-200 mg/dL [7.8-11.1 mmol/L] following standard OGTT) and/or hyperinsulinemia (fasting insulin > 15 μU/mL [104 pmol/L]) at the initial evaluation (see above) and were eligible to enroll in a 3 month double-blind randomized controlled trial of metformin (500 mg orally twice daily). Subjects were excluded from the study if they had a history of renal failure, serum creatinine > 1.5 mg/dL [132.6 μmol/L], history of congestive heart failure, elevated SGOT, alkaline phosphatase or prothrombin time > 2.0 x the upper limit of normal, hemoglobin < 8 mg/dL [<1.2 mmol/L], history of diabetes mellitus or diabetic response to OGTT (fasting glucose > 126 mg/dL [>7.0 mmol/L] or 2 hour blood glucose > 200 mg/dL [>11.1 mmo/L]), or pregnancy. In addition, subjects were discontinued from the study if they changed their antiviral regimen for any reason during the 3 month study period (n=1). Twenty-six subjects were randomized and the primary results of this study have been published previously (15).

Protocol: Eligible subjects were randomly assigned to receive either metformin (500 mg po twice daily, Bristol Myers Squibb, Inc. Plainsboro, NJ) or an identical placebo for three months. Randomization was stratified by sex and age (i.e. < 40 yrs and ≥ 40 yrs) and assigned in blocks of four based on randomly generated numbers. Randomization was performed by the MGH pharmacy. Investigators and subjects were blinded to drug assignment. Each subject completed a standard 75 gm OGTT following a 12 hour overnight fast on the day of randomization and three months following randomization. Serum insulin and glucose were determined at 0, 30, 60, 90 and 120 minutes and insulin AUC was calculated from these measures. In addition, at each visit, fasting blood samples were collected for determination of PAI-1 and tPA antigen levels.

Laboratory Methods: Insulin levels were measured in serum using radioimmunoassay (Diagnostic Product Corporation, Los Angeles, CA). Intra-assay and interassay coefficients of variation range from 4.7-7.7% and 5.5-9.2%, respectively. Cross reactivity with proinsulin at midcurve was at least 40%. HIV viral load was determined by the Amplicor HIV-1 Monitor Assay (Roche Molecular Systems, Branchburg, NJ) with a lower limit of detection of 400 copies/mL. Glucose was measured with a hexokinase reagent kit (Dade Dimension, Wilmington, DE). In the Framingham cohort, blood samples for tPA and PAI-1 were anticoagulated with 3.8% trisodium citrate (9:1 vol/vol). Blood samples for tPA and PAI-1 in HIV patients were anticoagulated with EDTA. The type of anticoagulant used is not known to influence ELISA results for PAI-1 or tPA. In all cases, samples were iced or centrifuged immediately, and plasma aliquots were stored at -70 to -80 °C until subsequent analysis. Levels of tPA antigen were determined by ELISA immunoassay (Biopool International, Ventura CA). The intra-assay CV was 5.5% and the interassay CV was 9.0%. PAI-1 was also determined by ELISA (Biopool International, Ventura CA) with interassay CV of 8.1% and intra-assay CV of 2.6%.

Statistical Analyses

Comparisons of clinical characteristics between HIV infected and control subjects were made using Student's t-test. We used a linear regression model including waist to hip ratio to control for the effects of regional fat distribution on differences between the groups. Stepwise linear regression was used to build a multivariate regression model with univariate predictors added to the model for p≤ 0.1. Among patients with HIV and fat redistribution, potential predictors of PAI-1 and tPA that were tested were age, BMI, gender, waist-to-hip ratio, smoking status, PI use, duration of HIV infection and insulin AUC. Analyses were repeated, stratified by gender. In the metformin treatment study, Student's t-test was used to assess baseline clinical characteristics between the two treatment groups. The treatment effect at three months was estimated using analysis of covariance. The measurement obtained at three months was the outcome variable, treatment assignment was the main effect and baseline measurement of the variable was used as a covariate. Two subjects (one in the placebo group and one in the metformin group) had PAI-1 levels at baseline which were significant outliers (greater than 2 standard deviations above the group mean) and therefore were excluded from the analyses of PAI-1 data. Statistical analyses were performed using SAS and SAS JMP (SAS Institute, Inc.Cary NC) and statistical significance was defined as two-tailed alpha value of P<0.05. All data are presented as mean (standard error of the mean).

Results

Cross-Sectional Comparison with Framingham Control Subjects

HIV infected patients and Framingham control subjects were matched on age, gender and BMI (Table 1). Ninety-nine percent (85/86) of patients were on an antiviral regimen containing an NRTI, 71% a PI and 27% an NNRTI. There was no difference between

Table II. Comparison of Metabolic and Hemostatic Parameter between Men and Women

	HIV-Infected Lipodystrophy		Control	
	Women (n=24)	Men (n=62)	Women (n=72)	Men(n=186)
Age (yr)	40 (2)*	44 (1)	41 (1)	45 (1)
BMI (kg/m^2)	26.0 (0.9)	26.2 (0.6)	26.1 (0.5)	26.2 (0.3)
WHR	0.96 (0.01)†	0.97 (0.01)‡	0.82 (0.01)	0.93 (.004)
Fasting Insulin				
(μU/mL)	15.4 (1.5)†	20.6 (2.4)‡	7.7 (0.8)	8.0 (0.6)
[pmol/L]	[107.0 (10.4)]†	[143.1 (16.7)]‡	[53.5 (5.6)]	[55.6 (4.1)]
2 hr Insulin				
(μU/mL)	112.5 (23.0)†	96.5 (11.1)‡	50.5 (4.9)	37.6 (2.9)
[pmol/L]	[781 (160)]†	[670 (77)]‡	[351 (34)]	[261 (20)]
PAI-1 (μg/L)	45.4 (3.1)†	46.4 (1.5)‡	18.0 (1.5)	19.3 (1.1)
tPA (μg/L)	12.8 (1.2)*†	18.0 (1.0)‡	7.1 (0.4)	8.4 (0.3)

† Women: HIV-infected vs Control, p value <0.01
‡ Men: HIV-infected vs Control, p value <0.01
* HIV Infected: Men vs Women, p value <0.05
WHR – waist-to-hip ratio, values shown are mean(SEM), [SI Units]

HIV-infected patients and controls in the prevalence of smoking. Sixty-five percent of the HIV infected patients presented with signs of both lipoatrophy and lipohypertrophy, 17% with primarily lipoatrophy and 17% with central lipohypertrophy without atrophy. There was no difference in PAI-1 or tPA antigen levels between these patient sub-types.

HIV infected patients with fat redistribution had significant elevations in fasting insulin, 2 hour OGTT insulin, PAI-1 and tPA antigen levels compared to control subjects. The differences in insulin concentration, PAI-1 and tPA between the two groups remained significant in a regression model controlling for waist-to-hip ratio (Table I). In a stepwise multivariate regression model of patients with HIV infection which included age, BMI, gender, smoking status, PI use, duration of HIV infection, insulin AUC, and waist-to-hip ratio, the only predictors of tPA that met the criteria for entry into the model were insulin AUC (P<0.0001), gender (P=0.0001) and duration of HIV infection (P=0.08). Using the same model strategy to assess predictors of PAI-1, there were no significant predictors of PAI-1 levels. Waist-to-hip ratio was the only significant predictor of PAI-1 in a univariate regression analysis (P=0.04) .

Analyses were repeated with stratification by gender (Table II). Both women and men with HIV and fat redistribution had increased levels of fasting and 2 hr OGTT insulin, PAI-1 and tPA compared to matched controls. PAI-1 and tPA remained significantly increased in women and men with HIV infection after adjusting for waist-to-hip ratio. Among the HIV patients, women were younger (P<0.05) and had lower levels of tPA compared to men (P<0.01). In a regression analysis of tPA levels among HIV patients, tPA levels remained significantly higher in men compared to women (P<0.05) after adjusting for age. There was no difference between men and women with HIV and fat redistribution in PAI-1 levels.

Patients in this initial cohort who were found to have impaired glucose tolerance (2-hour OGTT glucose between 140-200mg/dL [7.8-11.1 mmol/L]) or fasting hyperinsulinemia (>15 μU/mL [104 pmol/L]) were eligible for participation in a trial of metformin therapy. A multivariate regression model to assess predictors of impaired glucose tolerance (IGT) found that increasing age (P=0.05) and current PI use (P<0.05) predicted IGT, but BMI, gender, waist-to-hip ratio, duration of HIV infection, and smoking status did not predict IGT.

Response to Metformin Therapy

There were no significant differences in glucose, insulin, tPA and PAI-1 levels between patients randomized to placebo or metformin therapy at the baseline evaluation (all p-values > 0.05) (Table III). Patients randomized to receive metformin had significantly greater reductions in insulin AUC (-2930 ± 912 vs -414 ± 432 μU/mL*120 min; metformin vs placebo, P=0.02 [-20349 ± 6334 pmol/L vs 2875 ± 3000 pmol/L]), tPA (-1.9 ± 1.4 vs 1.4 ± 1.0 μg/L, P=0.02) and PAI-1 (-8.7 ± 2.3 vs 1.7 ± 2.9 μg/L, P=0.03) after three months of treatment compared to patients receiving placebo. Twelve weeks of metformin therapy was associated with an 11% reduction in tPA antigen level and a 16% reduction in PAI-1 levels (Figure 1). There was a positive linear correlation between the change in insulin AUC and the change in tPA (r=0.43, P=0.03). There was a trend towards significance for a correlation between change in insulin AUC and change in PAI-1 (r=0.35), but this was not statistically significant (P=0.1).

Discussion

In this study, we demonstrate increased concentrations of tPA and PAI-1, markers of impaired fibrinolysis, in HIV infected patients with fat redistribution compared to healthy age- BMI- and gender-matched control subjects. Elevated levels of tPA and PAI-1 were demonstrated in men and women with HIV infection and fat redistribution in association with hyperinsulinemia and central adiposity. The use of metformin was associated with significant reductions in tPA and PAI-1 concentrations.

Increased tPA antigen, a marker of impaired fibrinolysis, predicts increased risk of coronary artery disease mortality among patients with a history of angina pectoris and CVD (16), as well as cerebral vascular events among individuals without a prior history of cardiovascular disease (17). Further, there is a strong association between hyperinsulinemia and impaired glucose tolerance and levels of PAI-1 and tPA in otherwise healthy adults (10), suggesting a mechanistic link between insulin resistant states and increased CVD. We demonstrate that HIV infected individuals with fat redistribution have hyperinsulinemia and that this is associated with marked elevation in tPA. These patients also had significantly increased PAI-1 levels, which was associated with central fat distribution, another known characteristic of HIV lipodystrophy. While it is possible that PAI-1 and tPA levels may be elevated among HIV infected patients without evidence of fat redistribution as a part of HIV infection per se, our data demonstrate that increased hemostatic markers are directly related to central adiposity and hyperinsulinemia in this populatioin. Impaired fibrinolysis may significantly increase the risk of CVD in patients with HIV infection and fat redistribution.

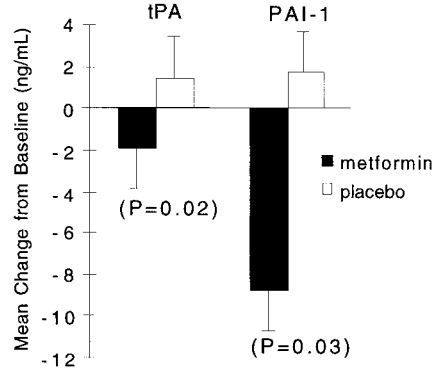

Figure 1. Mean change from baseline in tPA antigen and PAI-1 levels after 12 weeks of metformin vs placebo

Table III. Baseline and Mean Change after Three Months of Metformin vs Placebo: Insulin, Glucose, tPA and PAI-1

	Baseline		
	Placebo (n=11)	Metformin (n=14)	t-test p value
Age (y)	45.6 (1.9)	44.1 (2.1)	ns
Male/Female	8/3	11/3	ns
BMI (kg/m^2)	26.9 (1.1)	27.5 (1.2)	ns
CD4 cell count x10^6/L	535 (55)	486 (55)	ns
HIV Viral load (copies/mL)	6970 (6803)	1360 (1082)	ns
Fasting glucose (mg/dL)	95.5 (5.1)	87.9 (2.6)	ns
[mmol/L]	[5.3 (0.3)]	[4.9 (0.1)]	
Fasting insulin (µU/mL)	23.6 (3.3)	31.0 (8.8)	ns
[pmol/L]	[164 (23)]	[215 (61)]	
Glucose AUC mg/dL*120m	18004 (1046)	16795 (692)	ns
[mmol/L*120m]	[999 (58)]	[932 (38)]	
Insulin AUC µU/mL*120m	14146(2347)	14340(3333)	ns
[pmol/L*120m]	[9.8x10^4(16300)]	[9.9x10^4(23148)]	
tPA (µg/L)	18.5 (2.0)	16.7 (2.3)	ns
PAI-1 (µg/L)†	48.5 (3.9)	56.0 (3.5)	ns
	Change at 3 Months		
	Placebo (n=11)	Metformin (n=14)	ancova p-value
Fasting glucose (mg/dL)	1.9 (2.5)	-0.9 (2.1)	ns
[mmol/L]	[0.1 (0.1)]	[-0.05 (0.1)]	
Fasting insulin (µU/mL)	0.2 (2.8)	-4.3 (4.0)	ns
[pmol/L]	[1.4 (19.4)]	[-30.0 (27.8)]	
Glucose AUC mg/dL*120m	776 (496)	-563 (695)	ns
[mmol/L*120m]	[43.1 (27.5)]	[-31.2 (38.6)]	
Insulin AUC µU/mL*120m	-414 (432)	-2930 (912)	0.01
[pmol/L*120m]	[-2875 (3000)]	[-20349 (6334)]	
tPA (µg/L)	1.4 (1.0)	-1.9 (1.4)	0.02
PAI-1 (µg/L)†	1.7 (2.9)	-8.7 (2.3)	0.03

Values presented are unadjusted means (SEM), ns represents p>0.05, [SI Units]
Analysis of covariance (ancova), effect of treatment with baseline value as covariate
† PAI-1 levels n=10 placebo; n=13 metformin.

Among HIV-infected patients with fat redistribution and insulin resistance, 12 weeks of metformin therapy resulted in significant reductions in insulin AUC, PAI-1 and tPA levels. The reductions in tPA (11%) and PAI-1 (16%) were relatively small, but significant and achieved using low dose metformin over a short period of time. Further studies using higher doses over a longer duration will be necessary to determine the maximal effect of metformin on these indices of CVD risk.

Previous investigators have demonstrated reductions in PAI-1 and tPA following administration of metformin for type II diabetes (18) as well as reductions in tPA among non-diabetic obese patients (19). Our findings are consistent with these data and showed reductions in both PAI-1 and tPA in patients treated with metformin who have HIV- related lipodystrophy and insulin resistance. Hyperinsulinemia is a known independent marker of increased CVD risk, and elevated tPA and tPA/PAI-1 complex in association with hyperinsulinemia have been implicated as a causal mechanism in endothelial injury and potential thrombosis. Recent in vivo investigation in humans has demonstrated increased expression of PAI-1 and tPA following local infusion of insulin (20), further supporting a direct link between cardiovascular disease risk and insulin resistance. Central adipose tissue, which is increased among HIV infected patients with fat redistribution, also produces elevated levels of PAI-1 (21). Insulin resistance and central adiposity may place HIV infected patients with fat redistribution at substantial risk of CVD via impaired fibrinolysis. Metformin therapy and treatment with insulin sensitizing agents may reduce this risk by lowering insulin levels as well as other hemostatic markers, such as PAI-1 and tPA.

We found that men and women with HIV and fat redistribution have comparable elevations in PAI-1, but that tPA levels were greater among men. Further, adjustment for differences in waist-to-hip ratio between patients and control subjects did not significantly modify the differences in levels of PAI-1 and tPA for either gender. These data suggest that differences in body fat distribution only partially explain an increased CVD risk profile among HIV infected patients, especially in women. Other factors, including hyperinsulinemia may mediate increased levels of hemostatic markers.

Our data demonstrate impaired fibrinolysis in association with hyperinsulinemia and increased waist to hip ratio in patients with HIV infection and fat redistribution. We have previously shown that metformin significantly reduced insulin levels, weight, blood pressure and waist circumference in this population and now show a significant effect of low dose metformin to reduce tPA and PAI-1 antigen levels. These data suggest that metformin improves the overall cardiovascular risk profile in HIV infected patients with fat redistribution. Further studies are necessary to determine the mechanisms and clinical effects of impaired fibrinolysis in HIV-associated fat redistribution and the optimal treatment strategy for managing CVD risk in this emerging population of patients.

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References

1. Carr A, Samaras K, Thorisdottir A, Kaufman GR, Chisolm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor associated lipodystrophy, hyperlipidemia, and diabetes mellitus: a cohort study. *Lancet*. 1999;353:2093-99.
2. Hadigan C, Meigs JB, Corcoran C, et al. Metabolic abnormalities and cardiovascular disease risk factors in HIV-infected men and women with the lipodystrophy syndrome. *Clin Inf Dis*, In Press.
3. Carr A, Samaras K, Burton S, et al. A syndrome of peripheral lipodystrophy, hyperlipidemia and insulin resistance in patients receiving protease inhibitor therapy. *AIDS*. 1998;12:F51-F58.
4. Hadigan C, Miller K, Corcoran C, Anderson E, Basgoz N, Grinspoon S. Fasting Hyperinsulinemia and Changes in Regional Body Composition in HIV-Infected Women. *J Clin Endocrinol Metab*. 1999;84:1932-37.
5. Hadigan C, Corcoran C, Stanley T, Piecuch S, Klibanski A, Grinspoon S. Fasting hyperinsulinemia in HIV-infected men: relationship to body composition, gonadal function and protease-inhibitor use. *J Clin Endocrinol Metab*. 2000;85:35-41.
6. Vigouroux C, Gharakhanian S, Salhi Y, et al. Diabetes, Insulin resistance and Dyslipidemia in Lipodystrophic HIV-Infected Patient on Highly Active Antiretroviral Therapy (HAART). *Diabetes and Metabolism (Paris)*. 1999;25:225-232.
7. Walli RK, Herfort O, Michl GM, et al. Treatment with protease inhibitors associated with peripheral insulin resistance and impaired oral glucose tolerance in HIV-1 infected patients. *AIDS*. 1998;12:F167-F173.
8. Feskens EJ, Kromhout D. Glucose tolerance and the risk of cardiovascular disease: the Zutphen Study. *J Clin Epidemiol*. 1992;45:1327-34.
9. Howard G, Bergman R, Wagenknecht LE, et al. Ability of alternative indices of insulin sensitivity to predict cardiovascular risk: comparison with the "minimal model". Insulin reistance Atherosclerosis Study (IRAS) Investigators. *Annals of Epidemiology*. 1998;8:358-69.
10. Meigs JB, Mittleman MA, Nathan DM, et al., Hyperinsulinemia, hyperglycemia, and impaired hemostasis: The Framingham Offspring Study. *JAMA* 2000;283:221-228.
11. Lohman TG, Roche AF, Martorell R, eds. *Anthropometric Standardization Reference Manual*. Champaign, IL: Human Kinetic Books; 1988.
12. WHO Expert Committee on Diabetes Mellitus: Second Report. Geneva: World Health Organization; 1980.
13. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham Offspring Study. *Am J Epidemiol*. 1979;110:281-90.
14. Meigs JB, Nathan DM, Wilson PWF, Cupples LA, Singer DE. Metabolic Risk Factors Worsen Continuously Across the Spectrum of Nondiabetic Glucose Tolerance: The Framingham Offspring Study. *Ann Int Med*. 1998;128:524-33.
15. Hadigan C, Corcoran C, Basgoz N, Davis B, Sax P, Grinspoon S. Metformin in the treatment of HIV lipodystrophy syndrome: A randomized controlled trial. *JAMA* 2000;284:472-477.

16. Jansson JH, Olofsson BO, Nilsson TK. Predictive value of tissue plasminogen activator mass concentration on long-term mortality in patients with coronary artery disease: A 7-year follow-up. *Circulation* 1993;88(1):2030-34.
17. Johansson L, Jansson JH, Boman K, Nilsson TK, Stegmayr B, Hallmans G. Tissue plasminogen activator, plasminogen activator inhibitor-1 and tissue plasminogen activator/plasminogen activator inhibitor-1 complex as risk factors for the development of first stroke. *Stroke* 2000;31:26-32.
18. Grant PJ. The effects of high- and medium-dose metformin therapy on cardiovascular risk factors in patients with type II diabetes. *Diabetes Care* 1996;19(1):64-66.
19. Charles MA, Morange P, Eschwege E, Andre P, Vague P, Juhan-Vague I. Effect of weight change and metformin on fibrinolysis and the von Willebrand factor in obese subjects: the BIGPRO1 study. Biguanides and the prevention of the risk of obesity. *Diabetes Care* 1998;21(11):1967-72.
20. Carmassi F, Morale M, Ferrini L, et al., Local insulin infusion stimulates expression of plasminogen activator in normal subjects. *Am J Med* 1999;107:344-50.
21. Alessi MC, Peiretti F, Morange P et al., Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. *Diabetes* 1997;46(5):860-7.