

Sustained Benefits of Metformin Therapy on Markers of Cardiovascular Risk in Human Immunodeficiency Virus-Infected Patients with Fat Redistribution and Insulin Resistance

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The purpose of this study was to evaluate the metabolic and cardiovascular benefits of continued metformin therapy for HIV-infected patients with lipodystrophy. Eligible subjects who participated in the 3-month randomized study received an additional 6-month open label metformin treatment extension. Nineteen of the 25 potential subjects were eligible to receive open label metformin based on preestablished safety and efficacy criteria. Insulin and glucose response to oral glucose challenge, cardiovascular disease risk markers (e.g. tissue plasminogen activator), weight, and anthropometric measurements were the primary outcome measures. Continued treatment with metformin resulted in further significant

reductions in tissue plasminogen activator antigen levels ($P = 0.02$) and body mass index ($P = 0.03$). Reductions in insulin levels were sustained during the 6-month treatment extension. In addition, waist circumference decreased significantly in subjects continuing metformin treatment ($P = 0.01$). Metformin was well tolerated and no one discontinued treatment due to side effects. These data demonstrate a sustained benefit of metformin treatment to reduce hyperinsulinemia and certain markers of cardiovascular disease risk in patients with HIV infection and lipodystrophy. (*J Clin Endocrinol Metab* 87: 4611–4615, 2002)

INSULIN RESISTANCE, dyslipidemia, and fat redistribution are increasingly recognized among HIV-infected patients (1–5). Increased truncal fat and reduced peripheral fat are seen in association with hyperlipidemia and increased risk factors for cardiovascular disease (CVD) (5, 6). Short-term administration of metformin, an insulin-sensitizing agent, has been shown to improve insulin levels and to reduce central adiposity in patients with HIV infection and fat redistribution (7, 8). In a 3-month randomized placebo-controlled trial, we demonstrated that metformin (500 mg twice daily) reduced insulin levels, weight, and diastolic blood pressure among HIV-infected subjects with lipodystrophy and evidence of insulin resistance (7). Metformin treatment was also associated with reduction in the CVD risk markers, tissue plasminogen activator (tPA), and plasminogen activator inhibitor-1 (PAI-1) antigen concentrations (6). However, the benefits of prolonged metformin therapy in this population are unknown. To assess the efficacy of continued metformin therapy in HIV lipodystrophy, we investigated the effects of metformin during a 6-month open label extension among subjects who initially participated in the 3-month randomized metformin trial.

Subjects and Methods

Subjects

Detailed descriptions of the recruitment and eligibility criteria for the initial randomized trial have been reported previously (6, 7). Briefly,

Abbreviations: BMI, Body mass index; CVD, cardiovascular disease; DEXA, dual energy x-ray absorptiometry; OGTT, oral glucose tolerance test; PAI-1, plasminogen activator inhibitor-1; tPA, tissue plasminogen activator.

subjects were required to have fat redistribution documented on physical examination and have either fasting hyperinsulinemia (insulin, $>15 \mu\text{U}/\text{ml}$; $140 \text{ pmol}/\text{liter}$) or impaired glucose tolerance [oral glucose tolerance test (OGTT); 2 h glucose between $140\text{--}200 \text{ mg}/\text{dl}$ ($7.8\text{--}11.1 \text{ mmol}/\text{liter}$)]. Written informed consent was obtained from each subject before 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.

Protocol

After completion of the 3-month randomized trial, subjects were eligible to receive open label metformin treatment for 6 months if they met the following inclusion criteria. Subjects initially randomized to placebo began open label metformin if they continued to meet the original entry criteria outlined above. Subjects initially randomized to metformin (500 mg twice daily) were continued on metformin if glucose tolerance or fasting hyperinsulinemia improved 15% or greater after the first 3 months. Subject entry and flow-through the protocol is summarized in Fig. 1.

Subjects continuing metformin received 500 mg twice daily, but increased to 850 mg twice daily if glucose tolerance or insulin levels had not normalized (normalization of insulin level was defined as fasting insulin $<15 \mu\text{U}/\text{ml}$). Subjects initiating metformin therapy received 500 mg twice daily for the first 3 months of open therapy and increased to 850 mg twice daily thereafter if they met the criteria outlined above. Subjects completed follow-up visits 3 and 6 months after starting open label treatment, which included a standard 75-g OGTT, fasting determination of tPA antigen, PAI-1 antigen levels, lipid concentrations, lactate, CD4 count, and HIV viral RNA levels. Weight, blood pressure, and hip and waist circumferences were measured at each visit. Subjects were not excluded from open treatment if they changed antiviral regimens or initiated hormone replacement therapy, but information about medication changes was recorded at each visit. PAI-1 and tPA antigen levels were determined by ELISA immunoassay (Biopool International, Ventura, CA). The tPA intraassay coefficient of variation (CV) was 5.5%, and the interassay CV was 9.0%. The PAI-1 interassay CV was 8.1%, and the intraassay CV was 2.6% (6).

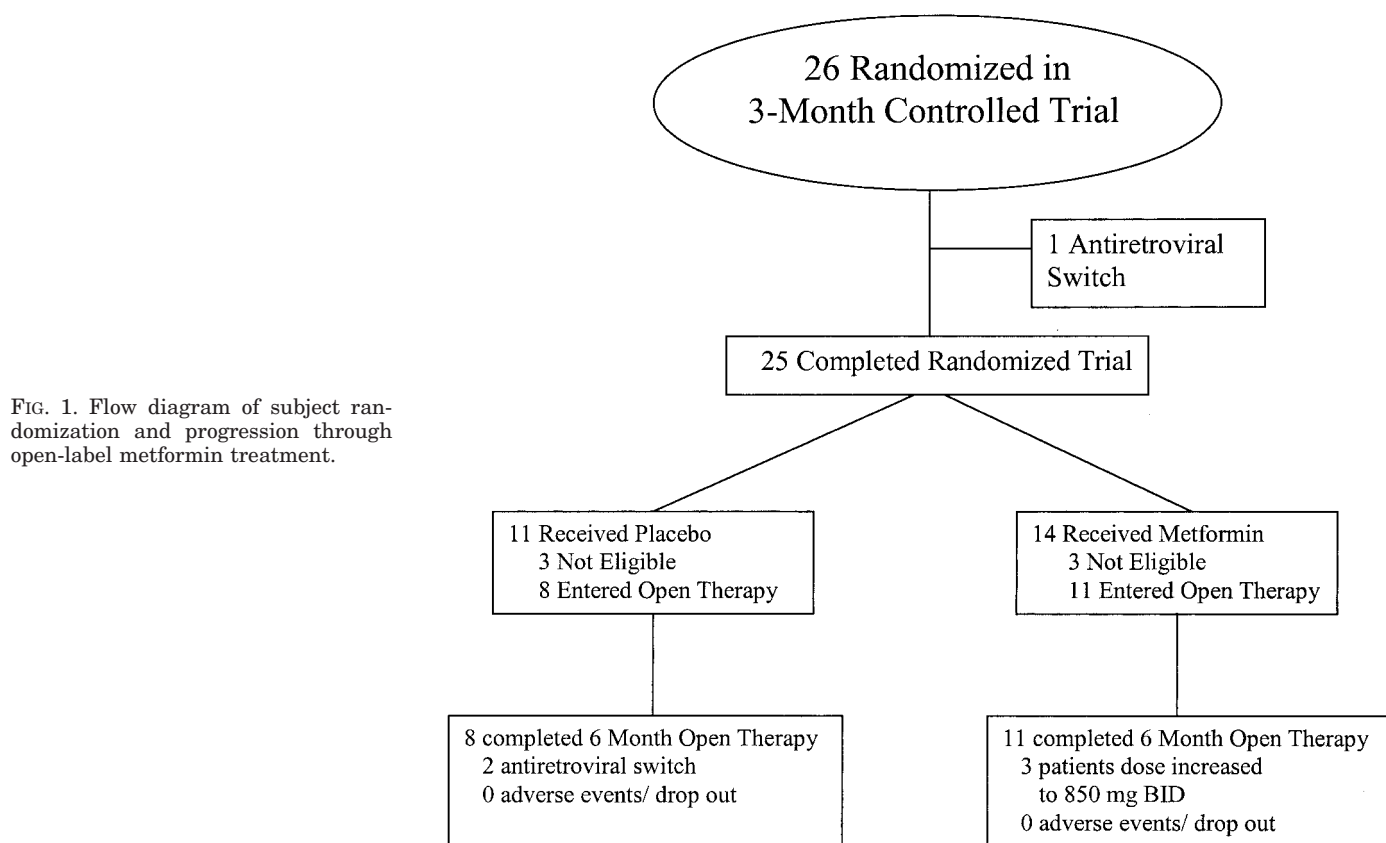


FIG. 1. Flow diagram of subject randomization and progression through open-label metformin treatment.

Body fat distribution was determined by dual energy x-ray absorptiometry (DEXA) using a QDR-4500A scanner (Hologic, Inc., Bedford, MA). Regions of interest, namely arms, legs, and trunk, were standardized (1995 Users Guide, Hologic, Inc.). The DEXA scan was used to determine total body fat, and regional percent body fat, such as truncal percent fat (trunk fat grams per total trunk mass) and extremity percent fat (sum of four extremities fat grams per total sum of four extremities mass). Laboratory methods were outlined previously (6, 7).

Statistical analysis

The primary outcome variables of this study were tPA, PAI-1, insulin, and glucose AUC following standard OGTT. Secondary end points included lipid concentrations, body mass index (BMI), waist to hip ratio, waist circumference, and fat distribution as determined by DEXA. Paired *t* tests within each group were used to compare outcome variables before and after the 6-month open label treatment. The sustained effects of metformin on tPA were confirmed using a longitudinal random effects model (data not shown) (9). Statistical analyses were performed using SAS JMP (SAS Institute, Inc., Cary, NC), and statistical significance was defined as a two-tailed α level of $P < 0.05$. All data are presented as the mean \pm SEM.

Results

Twenty-five subjects completed the 3-month randomized trial of metformin therapy; 11 of 14 subjects originally assigned to metformin, and 8 of 11 subjects initially assigned to placebo completed 6 months of open metformin therapy (Fig. 1 and Table 1). Three subjects increased to 850 mg, orally, twice daily; all other participants continued on 500 mg twice daily for the remainder of the study. No subject was discontinued or dropped out of the open treatment portion of the study.

TABLE 1. Clinical characteristics at the beginning of open label treatment

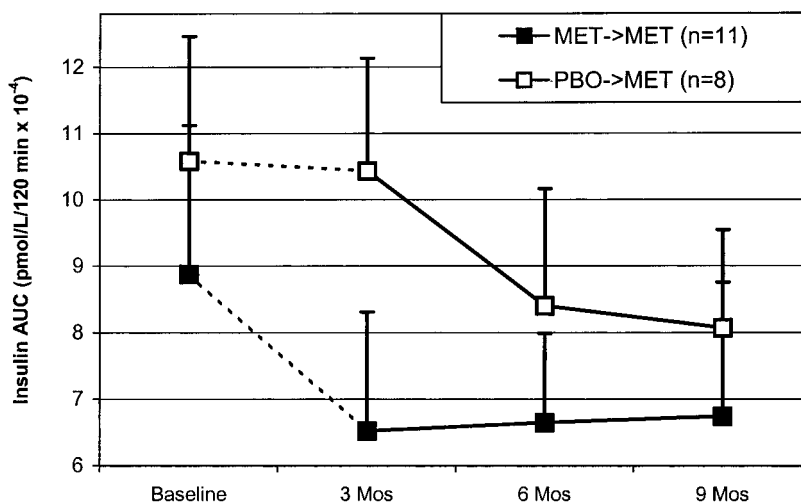
Initial randomization	Metformin (n = 11)	Placebo (n = 8)	P
Male/female	10/1	7/1	NS
Age (yr)	44.2 \pm 2.5	46.9 \pm 2.2	NS
Duration of HIV (yr)	5.0 \pm 0.8	7.3 \pm 1.7	NS
Current PI use (%)	100%	75%	NS
Duration of PI (months)	25.9 \pm 4.2	29.5 \pm 3.1	NS
HIV RNA (copy/ml)	1714 \pm 1371	1138 \pm 537	NS
CD4 count (cells/mm ³)	477 \pm 54	480 \pm 34	NS

Values shown are mean \pm SEM. PI, Protease inhibitor; NS, not significant.

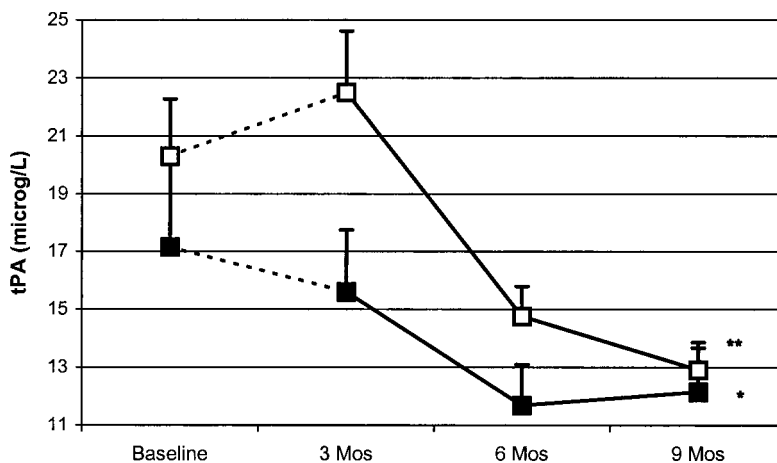
Effect of metformin therapy

Initiation of metformin treatment was associated with significant reductions in tPA levels ($P = 0.002$) and continued therapy led to further significant decreases in tPA concentrations ($P = 0.02$; Fig. 2). The initial reduction in PAI-1 among those initially randomized to metformin was sustained, and concentrations decreased slightly in both groups during open treatment, but this reduction was not significant. However, BMI decreased significantly for both subject groups over the 6-month open label treatment ($P < 0.05$ for initiators and $P = 0.03$ for continued treatment). Improvement in insulin AUC among subjects continuing metformin therapy for 6 months was sustained (*i.e.* there was no significant difference between the beginning and end of open treatment; $P = 0.8$; Fig. 2 and Table 2).

A Insulin Area Under the Curve (AUC) in Response to Metformin



B tPA Response to Metformin



C PAI-1 Response to Metformin

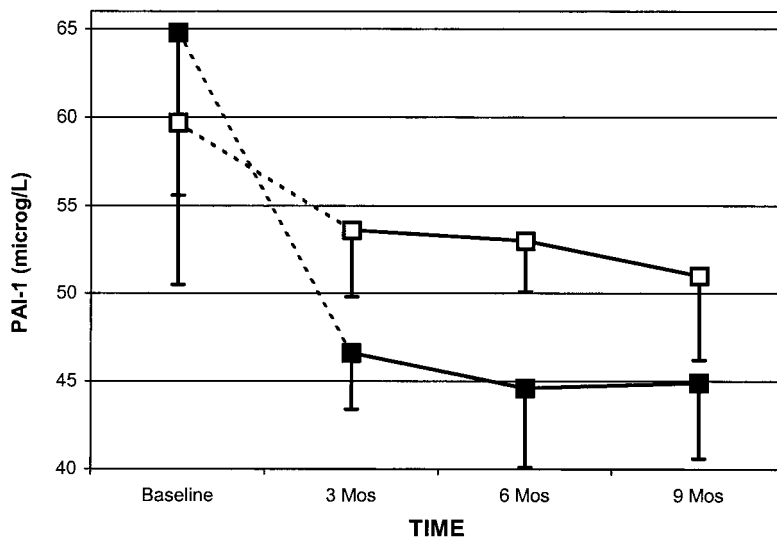


FIG. 2. Mean insulin area under the curve (AUC) after OGTT (A) and tPA antigen (B) and PAI-1 antigen (C) concentrations. □, Subjects initially randomized to placebo (PBO; n = 8) who started metformin (MET) at the 3 month visit. ■, Subjects initially randomized to metformin who continued on open therapy (n = 11). ---, The double-blind portion of the study; —, open label treatment. By paired *t* test comparing 3 months to 9 months in each group: **, *P* = 0.002; *, *P* = 0.02.

TABLE 2. Metabolic and anthropometric measurements during open metformin treatment

	Initially randomized to metformin (n = 11)		Initially randomized to placebo (n = 8)	
	Pre-open treatment	9 months	Pre-open treatment	9 months
Fasting glucose (mmol/liter)	4.9 ± 0.2	4.9 ± 0.1	5.7 ± 0.3	5.4 ± 0.2
Fasting insulin (pmol/liter)	174 ± 47	142 ± 28	184 ± 33	160 ± 24
Glucose AUC (mmol/liter·120 min)	834 ± 37	835 ± 38	1,124 ± 70	1,041 ± 57
Insulin AUC (pmol/liter·120 min)	65,186 ± 17,883	67,443 ± 20,092	104,342 ± 16,932	80,715 ± 14,703
tPA (μg/liter)	15.6 ± 2.1	12.2 ± 1.5 ^a	22.5 ± 2.1	12.9 ± 0.9 ^b
PAI-1 (μg/liter)	46.6 ± 3.2	44.9 ± 4.3	53.6 ± 3.9	51.1 ± 4.8
Cholesterol (mmol/liter)	6.5 ± 0.3	6.3 ± 0.4	6.4 ± 0.6	6.0 ± 0.4
LDL (mmol/liter)	3.4 ± 0.5	3.5 ± 0.2	4.3 ± 0.8	3.7 ± 0.5
HDL (mmol/liter)	1.1 ± 0.1	1.0 ± 0.1	1.3 ± 0.3	0.9 ± 0.1
Triglyceride (mmol/liter)	4.86 ± 0.84	3.19 ± 1.48	5.53 ± 1.93	6.32 ± 2.29
Lactic acid (mmol/liter)	2.5 ± 0.3	2.3 ± 0.3	1.4 ± 0.1	1.5 ± 0.2
BMI (kg/m ²)	28.2 ± 1.2	27.6 ± 1.1 ^a	27.8 ± 1.5	27.3 ± 1.4 ^a
Waist/hip ratio	1.00 ± .01	0.98 ± .01	1.00 ± 0.01	0.99 ± .02
Waist circumference (cm)	98.2 ± 3.0	96.1 ± 2.6 ^a	98.9 ± 4.2	97.4 ± 4.1
Total fat mass (kg)	19.4 ± 2.1	18.6 ± 2.0 ^c	18.8 ± 3.0	18.2 ± 2.7
Extremity fat mass (kg)	6.3 ± 0.8	5.8 ± 0.8 ^a	6.2 ± 1.2	5.9 ± 1.1
Extremity % fat	17.4 ± 1.6	16.7 ± 1.9	16.7 ± 2.8	16.5 ± 2.5
Trunk % fat	26.3 ± 1.6	26.0 ± 1.6	25.3 ± 2.9	24.7 ± 2.6
Diastolic BP (mm Hg)	73 ± 3	77 ± 3	81 ± 2	77 ± 1

P values for pre- vs. post-open treatment represent effect of metformin using paired *t* test result within each group: ^a *P* < 0.05, ^b *P* < 0.01, ^c *P* = 0.05. LDL, Low-density lipoprotein; HDL, high-density lipoprotein; BP, blood pressure.

Waist circumference decreased significantly for subjects continuing treatment (*P* = 0.01), and there was a trend for a reduction among subjects initiating treatment (*P* = 0.07). There was no significant reduction in trunk or extremity percent fat during open treatment, but total fat mass (*P* = 0.05) and extremity fat mass (*P* = 0.04) declined for subjects continuing treatment. There was no significant effect of metformin therapy on cholesterol, low density lipoprotein, high density lipoprotein, or triglyceride levels. There was a non-significant trend toward decreased cholesterol (*P* = 0.09) and decreased low density lipoprotein (*P* = 0.09) for subjects receiving open treatment who were originally given placebo. There was no effect of metformin on CD4 cell count or HIV viral load (data not shown).

Two subjects changed antiretroviral regimens; one discontinued indinavir and started efavirenz 7 wk before completion, and one discontinued efavirenz and started abacavir and nevirapine 2 wk after starting metformin therapy. In addition, one subject initiated testosterone replacement therapy (5 mg transdermal patch/d) 1 month before completion. Four subjects were taking a lipid-lowering agent throughout the study. One subject started using atorvastatin after completing 6 months of metformin therapy.

Side effects, safety, and adherence

Metformin was well tolerated. Fifty percent of the subjects initiating metformin therapy reported mild diarrhea that resolved by the 6-month visit in all but two subjects. Thirty-six percent of the subjects continuing metformin therapy reported mild diarrhea, which resolved in all subjects. There were no serious adverse events, and no one discontinued medication due to side effects. No subject developed lactic acidosis. Medication compliance was 93% based on returned unused study drug.

Discussion

We demonstrate a sustained benefit of metformin therapy to reduce indexes of insulin resistance and markers of cardiovascular disease risk in patients with HIV infection and fat redistribution. After a 6-month open label treatment extension, subjects demonstrated significant continued reductions in tPA antigen levels and BMI as well as sustained benefits in insulin area under the curve. Among subjects who were initial responders to metformin, the improvement in insulin AUC seen in the 3-month randomized trial was sustained after a total of 9 months. Furthermore, our data suggest that prolonged administration of metformin is well tolerated and associated with improvement in indexes of CVD risk (*i.e.* insulin, BMI, waist circumference, and tPA).

Due to concerns for patient safety, the total sample size and the number of subjects who received dose increases were limited by adherence to previously established eligibility criteria. Despite the small sample size and the conservatively low dose used in most subjects, markers of CVD risk, insulin resistance, and central adiposity were improved after the 6-month open label treatment extension.

Increased levels of tPA and PAI-1 have been associated with increased risk of CVD (10, 11); therefore, it is possible that reduction in these markers with the use of metformin may improve the CVD risk profile in HIV-infected patients with fat redistribution. Longitudinal CVD outcome studies are needed to determine whether there is an increased incidence of CVD in this population and whether risk modification will be able to alter cardiovascular health. The beneficial effect of metformin on BMI and waist circumference not only persisted, but continued to improve during the 6-month treatment extension. There was an overall reduction in BMI, and total fat mass was reduced in patients continuing metformin therapy. In this group there was a similar reduction in extremity fat mass. These preliminary data suggest that fat loss associated with metformin treatment may be

uniformly distributed. Metformin may therefore be most appropriate for patients with insulin resistance and truncal adiposity, as opposed to patients who have predominantly peripheral lipotrophy in whom further overall weight loss and decreased extremity fat are not desirable.

There was no significant effect of metformin on lipid levels in either the 3-month controlled trial or the 6-month extension. Metformin has been associated with modest reductions in cholesterol and triglyceride levels in patients with type II diabetes mellitus (12, 13). The inability to show a reduction in lipid levels in the present study may be attributable to the relatively low dose of metformin used. We did not exclude patients already receiving lipid-lowering medication, and this may have limited our ability to demonstrate an additional benefit from metformin. Furthermore, the hyperlipidemia associated with HIV and fat redistribution may be partially due to direct effects of antiretroviral therapy that are not modifiable by metformin. Nonetheless, persistent hyperlipidemia may contribute to increased CVD risk in this population.

This study demonstrates that the benefits of metformin on hyperinsulinemia, CVD risk marker (tPA antigen), weight, and waist circumference were sustained in subjects who completed 6–9 months of therapy. Furthermore, additional reductions in tPA antigen levels, BMI, and waist circumference were noted with continued therapy. Metformin was well tolerated and was not associated with serious adverse events. Subjects in this study were carefully screened for preexisting impairment in liver function, and therefore our results may not be generalized to other HIV-infected populations. Future investigation is necessary to determine the potential benefits of higher doses of metformin and to evaluate the ability of metformin to mediate the development of CVD or type II diabetes mellitus in patients with metabolic complications of HIV infection and fat redistribution.

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References

1. Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, Cooper D 1998 A syndrome of peripheral lipodystrophy, hyperlipidemia and insulin resistance in patients receiving protease inhibitor therapy. *AIDS* 12:F51–F58
2. Saint-Marc T, Partisani M, Poizat-Martin I, Bruno F, Rouviere O, Lang JM, Gastaut JA, Touraine JL 1999 A syndrome of peripheral fat wasting (lipodystrophy) in patients receiving long-term nucleoside analogue therapy. *AIDS* 13:1659–1667
3. Gervasoni C, Ridolfo AL, Trifirò G, Santabrogio S, Norbiato G, Musicco M, Clerici M, Galli M, Moroni M 1999 Redistribution of body fat in HIV-infected women undergoing combined antiretroviral treatment. *AIDS* 13:465–471
4. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA 1999 Diagnosis, prediction and natural course of HIV-1 protease inhibitor associated lipodystrophy, hyperlipidemia, and diabetes mellitus: a cohort study. *Lancet* 353:2093–2099
5. Hadigan C, Meigs JB, Corcoran C, Rietschel P, Piecuch S, Basgoz N, Davis B, Sax P, Stanley T, Wilson PW, D'Agostino RB, Grinspoon S 2001 Metabolic abnormalities and cardiovascular disease risk factors in adults with human immunodeficiency virus infection and lipodystrophy syndrome. *Clin Infect Dis* 32:130–139
6. Hadigan C, Meigs JB, Rabe J, D'Agostino RB, Wilson PW, Lipinska I, Tofler GH, Grinspoon S 2001 Increased PAI-1 and tPA antigen levels are reduced with metformin therapy in HIV infected patients with fat redistribution and insulin resistance. *J Clin Endocrinol Metab* 86:939–943
7. Hadigan C, Corcoran C, Basgoz N, Davis B, Sax P, Grinspoon S 2000 Metformin in the treatment of HIV lipodystrophy. *JAMA* 284:472–477
8. Saint-Marc T, Touraine JL 1999 Effects of metformin on insulin resistance and central adiposity in patients receiving effective protease inhibitor therapy. *AIDS* 13:1000–1002
9. Laird NM, Ware JM 1982 Random effects models for longitudinal data. *Biometrics* 30:963
10. Jansson JH, Olofsson BO, Nilsson TK 1993 Predictive value of tissue plasminogen activator mass concentration on long-term mortality in patients with coronary artery disease: a 7-year follow-up. *Circulation* 88:2030–2034
11. Johansson L, Jansson JH, Boman K, Nilsson TK, Stegmayr B, Hallmans G 2000 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* 31:26–32
12. DeFronzo RA, Goodman AM 1995 Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. *N Engl J Med* 333:541–549
13. Robinson AC, Burke J, Robinson S, Johnston DG, Elkeles RS 1998 The effects of metformin on glycemic control and serum lipids in insulin treated NIDDM patients with suboptimal metabolic control. *Diabetes Care* 21:701–705