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Blunted lipolysis and fatty acid oxidation during moderate exercise in HIV-infected subjects taking HAART

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Abstract

The protease inhibitor (PI) ritonavir (RTV) has been associated with elevated resting lipolytic rate, hyperlipidemia, and insulin resistance/glucose intolerance. The purpose of this study was to examine relationships between lipolysis and fatty acid (FA) oxidation during rest, moderate exercise and recovery, and measures of insulin sensitivity/glucose tolerance and fat redistribution in HIV-positive subjects taking RTV ($n = 12$), HAART but no PI ($n = 10$), and HIV-seronegative controls ($n = 10$). Stable isotope tracers [$1\text{-}^{13}\text{C}$]palmitate and [$1,1,2,3,3\text{-}^2\text{H}_5$]glycerol were continuously infused with blood and breath collection during 1-h rest, 70-min submaximal exercise ($50\% \dot{V}_{\text{O}_2 \text{ peak}}$), and 1-h recovery. Body composition was evaluated using DEXA, MRI, and MRS, and 2-h oral glucose tolerance tests with insulin monitoring were used to evaluate glucose tolerance and insulin resistance. Lipolytic and FA oxidation rates were similar during rest and recovery in all groups; however, they were lower during moderate exercise in both HIV-infected groups [glycerol R_a : HIV + RTV 5.1 ± 1.2 vs. HIV + no PI 5.9 ± 2.8 vs. Control 7.4 ± 2.2 $\mu\text{mol}\cdot\text{kg}$ fat-free mass (FFM) $^{-1} \cdot \text{min}^{-1}$; palmitate oxidation: HIV + RTV 1.6 ± 0.8 vs. HIV + no PI 1.6 ± 0.8 vs. Control 2.5 ± 1.7 $\mu\text{mol}\cdot\text{kg}$ FFM $\cdot\text{min}$, $P < 0.01$]. Fasting and orally-challenged glucose and insulin values were similar among groups. Lipolytic and FA oxidation rates were blunted during moderate exercise in HIV-positive subjects taking HAART. Lower FA oxidation during exercise was primarily due to impaired plasma FA oxidation, with a minor contribution from lower nonplasma FA oxidation. Regional differences in adipose tissue lipolysis during rest and moderate exercise may be important in HIV and warrant further study.

Keywords

human immunodeficiency virus; highly active antiretroviral therapy; ritonavir; lipodystrophy; insulin resistance; metabolic syndrome; mass spectrometry

Treatment of the human immunodeficiency virus (HIV-1) with highly active antiretroviral therapy (HAART) has dramatically reduced morbidity and mortality (27). However, HIV infection and HAART have been associated with several undesirable side effects that occur in 40–60% of HIV-infected people, including insulin resistance/glucose intolerance,

diabetes, hyperlipidemia, hypercholesterolemia, and adipose tissue redistribution (visceral adiposity and/or peripheral lipoatrophy). These comprise a cluster of metabolic alterations referred to as HIV-lipodystrophy (7, 34, 47).

Recent evidence (12, 36, 44) indicates that basal whole body lipolytic rate, intramyocellular lipid (IMCL), and hepatic lipid content are elevated in people with HIV-lipodystrophy who take protease inhibitor (PI)-including HAART. Although controversial (36), resting whole body fatty acid (FA) oxidation appears to be decreased in HIV-infected individuals with metabolic complications (22) and may be the result of HIV-and/or nucleoside analog reverse transcriptase inhibitor-associated mitochondrial toxicity (21). Ritonavir (RTV; Norvir), a PI, is commonly prescribed as part of HAART in the treatment of HIV infection. Ritonavir has been associated with hypertriglyceridemia (37), due in part to an increased rate of resting lipolysis (1), and may induce insulin resistance (20). In vitro studies (6, 24) have found that PIs decrease sterol regulatory element-binding protein-1 activation, a positive regulator of the adipocyte differentiation transcription factor peroxisome proliferator-activated receptor- γ (PPAR γ), thereby inhibiting its actions. It has been suggested that PI-mediated inhibition of PPAR γ (3) may contribute to an increased resting lipolytic rate (11) and eventually to peripheral lipoatrophy (3).

On the basis of the alterations in resting FA metabolism, we hypothesized that exercise-induced lipolysis would be increased and FA oxidation decreased in HIV-infected subjects taking HAART and more so in those taking RTV. We further hypothesized that these defects in FA metabolism during rest and exercise would be associated with higher muscle and hepatic lipid content and insulin resistance in these individuals.

RESEARCH DESIGN AND METHODS

Subjects

HIV-infected and HIV-seronegative men and women were recruited from the AIDS Clinical Trials Unit, the Infectious Disease Clinics, and the Volunteers for Health Program at Washington University School of Medicine and from the surrounding community. Volunteers received a physical examination, including a medical history, fasting blood chemistry, complete blood cell count, plasma HIV RNA quantitation (Roche Amplicor HIV-1 Monitor; Roche Diagnostics, Indianapolis, IN), and a 2-h oral glucose tolerance test with insulin monitoring. Volunteers were excluded if they were taking medications or dietary supplements that affect lipid or glucose metabolism or peak heart rate (i.e., statin, fibrate, sulfonylurea, corticosteroids, β -adrenergic blockers). All subjects consumed less than three alcohol-containing beverages per week, reported not using recreational drugs for 6 mo prior to enrollment, and were weight stable (<2% weight change in the 3 mo prior to the study). None of the subjects participated regularly (more than twice/wk) in exercise activities that would constitute exercise training. Eligible subjects were assigned to one of three groups: 1) HIV-seropositive subjects presently taking HAART, including the PI RTV (HIV + RTV, $n = 12$); 2) HIV-seropositive subjects taking HAART, not including a PI (HIV + no PI, $n = 10$); and 3) HIV-seronegative controls matched for age, gender, and activity level (Control, $n = 10$). Time since known HIV infection was 98 ± 67 mo for HIV + RTV and 105 ± 66 mo for HIV + no PI. All HIV-infected subjects had plasma HIV RNA levels <1,000 copies/ml, except for one subject in the HIV + RTV group (HIV RNA = 15,400 copies/ml), and none had an opportunistic infection or CD4 count <200 cells/ μ l (median CD4: HIV + RTV 345 cells/ μ l, HIV + no PI 558 cells/ μ l, $P = 0.03$). HIV-infected subjects were stable (<6 mo) on their present anti-HIV regimens (time on HAART: HIV + RTV 37 ± 25 mo, HIV + no PI 84 ± 30 mo, $P = 0.01$), and HIV + RTV subjects had been taking RTV for 6 mo (mean: 15 ± 11 mo). HAART included the following medications: HIV + RTV: lopinavir (LPV)/RTV (Kaletra: 400 mg, RTV: 100 mg BID, $n = 7$), boosted RTV (100 mg

qd, $n = 5$), atazanavir (AZT; 300 mg qd, $n = 5$), tenofovir (300 mg qd, $n = 5$), combivir (3TC: 150 mg; AZT: 300 mg BID, $n = 1$), tenofovir/emtricitabine (TDF: 300 mg; FTC: 200 mg, $n = 4$), didanosine (200 mg qd, $n = 3$), lamivudine (300 mg qd, $n = 1$), nevirapine (200 mg BID, $n = 3$), and enfuvirtide (90 mg SQ BID, $n = 1$); HIV + no PI: abacavir/zidovudine/lamivudine (ABC: 300 mg; 3TC: 150 mg; AZT: 300 mg BID, $n = 4$), combivir (3TC: 150 mg; AZT: 300 mg BID, $n = 4$), tenofovir/emtricitabine (TDF: 300 mg; FTC: 200 mg, $n = 2$), didanosine (200 mg qd, $n = 1$), and lamivudine (300 mg qd, $n = 3$), efavirenz (600 mg qd, $n = 5$), and nevirapine (200 mg BID, $n = 3$). Those not taking PI (HIV + no PI) had no previous exposure to any PI except for one subject who had not taken PI in >1 year prior to enrollment. The Human Studies Committee at Washington University School of Medicine approved the study, and all subjects provided informed consent before participating.

Body Composition Assessment

Whole body, trunk and limb fat mass (FM), and fat-free mass (FFM) were quantified using a Hologic Discovery (version 12.4; Waltham, MA) enhanced-array dual-energy X-ray absorptiometer (DEXA). Fat distribution was described as a ratio: trunk FM to limb FM.

Thigh subcutaneous fat and abdominal [subcutaneous (SAT) and visceral adipose tissue (VAT)] fat areas were quantified using proton magnetic resonance imaging (Siemens, Iselin, NJ). For the thigh, eight serial axial images were obtained in a region 10–25 cm above the superior border of the medial condyle of the tibia. SAT and VAT were identified in eight serial axial images obtained at the level of the L₂–L₃ interspace (level of the kidneys). Fat areas were identified and quantified (cm²) using Analyze Direct software (Rochester, MN). Total abdominal adipose tissue (TAT) area was calculated as the sum of SAT and VAT. Intrahepatic and IMCL lipid content were quantified using proton magnetic resonance spectroscopy (1.5 T whole-body system, Magnetom Sonata; Siemens, Erlangen, Germany) (23).

Peak Exercise Test

Each subject's peak oxygen consumption ($\dot{V}O_{2\text{ peak}}$) was measured on an electronically braked cycle ergometer (SensorMedics, Yorba Linda, CA). The work rate started at 20 W and increased 20 W every minute until volitional exhaustion. Continuous ECG (Quinton, Bothell, WA) and metabolic data (ParvoMedics TrueOne, Sandy, UT) were obtained. Exercise tests were considered maximal if peak heart rate reached 85% of that predicted for age ($220 - \text{age}$) and/or peak respiratory exchange ratio > 1.15 (2).

Lipid Metabolism Study

Subjects were admitted to the General Clinical Research Center at Washington University at 1700 the night before the lipid metabolism study. At 1800, subjects were given a standardized meal containing 12 kcal/kg body wt and 55% carbohydrate, 30% fat, and 15% protein. At 1900, subjects ingested a high-carbohydrate liquid beverage (80 g carbohydrates, 12.2 g fat, 17.6 g protein) (Ensure; Ross Laboratories, Columbus, OH) to ensure adequate muscle and hepatic glycogen stores. Subjects then fasted overnight and until completion of the study the following day.

At 0600 the following morning, catheters were inserted into a forearm vein for isotope infusion and into a contralateral hand vein (heated to 55°C) for arterialized venous sampling. At 0800 (time 0 min), a priming dose of NaH¹³CO₂ (1.5 $\mu\text{mol/kg}$) dissolved in 0.9% saline was administered, and constant infusions of [1-¹³C]palmitate (0.035 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) bound to 25% serum albumin (Centeon; LLC, Kankakee, IL) and [1,1,2,3,3-²H₅]glycerol (0.12 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) dissolved in 0.9% saline were started and maintained for the entire study period (190 min). All tracers were obtained from Cambridge Isotope Laboratories

(Andover, MA). Subjects rested from 0800 to 0900 (rest period, $t = 0 - 60$ min). At 0900, subjects transferred from the bed onto the cycle ergometer and exercised at a work rate consistent with 50% of their measured $\dot{V}O_{2\text{ peak}}$ and exercised for 70 min (exercise period, $t = 60 - 130$ min). Following completion of the exercise bout, subjects transferred back into the semirecumbent position on the bed and rested for 60 min (recovery period, $t = 130 - 190$ min).

Blood samples were obtained prior to the start of isotope infusions every 15 min during the rest and recovery periods and every 10 min during the exercise period to determine glycerol and palmitate tracer-to-tracee ratios (TTRs) and plasma substrate and hormone concentrations. Blood samples for FA and glycerol concentrations were collected into vials containing EDTA, those for hormone concentrations were collected into vials containing both EDTA and trasylol, and those for catecholamine analysis were collected into vials containing reduced glutathione and EGTA. Blood samples were placed on ice, and plasma was separated by centrifugation within 30 min of collection and stored at -70°C until analysis. Breath samples to determine $^{13}\text{CO}_2$ breath enrichment were collected in evacuated tubes at the same time intervals as the blood samples (25). Whole body oxygen consumption and $\dot{V}O_{2\text{ peak}}$ production were measured at baseline ($t = -15$ min) (SensorMedics; Deltatrac, Yorba Linda, CA) every 15 min during the exercise period and every 15 min during the recovery period (ParvoMedics TrueOne).

Acetate Infusion Study

All subjects completed an acetate infusion study 1 wk prior to the lipid metabolism study to determine the acetate correction factor needed to calculate plasma free fatty acid (FFA) oxidation (35). The acetate infusion protocol was identical to the lipid metabolism study, except that ^{13}C -labeled acetate was infused instead of the palmitate and glycerol tracers. Briefly, at 0800, a priming dose of $\text{NaH}^{13}\text{CO}_3$ ($1.5 \mu\text{mol/kg}$) dissolved in 0.9% saline was administered, and a constant infusion of $[1-^{13}\text{C}]$ sodium acetate ($0.035 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), dissolved in 0.9% saline, was started and maintained until the end of the study period (190 min). Blood and breath samples were collected as described for the lipid metabolism study.

Sample Analyses

Plasma glucose concentration was quantified using an automated glucose analyzer (Yellow Spring Instruments, Yellow Springs, OH). Plasma insulin and C-peptide concentrations were quantified using radioimmunoassay (13). Fasting serum lipids were quantified as described previously (46). Serum catecholamine concentrations were determined by an isotope-derivative radioenzymatic method (38). Blood lactate levels were quantified using a lactate dehydrogenase-based spectrophotometric assay (COBAS Mira; Roche Diagnostics, Basel, Switzerland). Plasma FFA concentrations were quantified by gas chromatography (Hewlett-Packard 5890-II; Hewlett-Packard, Palo Alto, CA) after heptadecanoic acid was added to plasma as an internal standard (29). Plasma glycerol concentrations were determined by gas chromatography-mass spectrometry (GC-MS) after $[2-^{13}\text{C}]$ glycerol was added to plasma as an internal standard (28).

The TTRs for plasma $[1-^{13}\text{C}]$ palmitate and $[1,1,2,3,3-2\text{H}_5]$ glycerol were determined by capillary GC-MS (Agilent 5973 Mass Selective Detector) as previously described (29). For $[1-^{13}\text{C}]$ palmitate and $[1,1,2,3,3-2\text{H}_5]$ glycerol quantitation, precipitation of plasma proteins and extraction of plasma lipids were performed through the addition of cold acetone and hexane, respectively. FFA were derivatized by conversion to their methyl esters with iodomethane and isolated by solid-phase extraction columns. $[1-^{13}\text{C}]$ Palmitate enrichment was quantified using GC-electron impact (EI) ionization-MS with selective ion monitoring [mass-to-charge ratio (m/z) 270.2 and 271.2]. The aqueous phase was dried by Speed-Vac

centrifugation (Savant Instruments, Farmingdale, NY), and glycerol was derivatized by addition of heptafluorobutyric anhydride. [1,1,2,3,3- $^2\text{H}_5$]Glycerol enrichments were determined by EI ionization mode (m/z 253, 254, and 257) and selectively monitored. Breath $^{13}\text{CO}_2$ enrichment (m/z 45/44) was quantified using gas isotope ratio MS (IRMS; Finnigan Delta+ XL-IRMS, Bremen, Germany) as previously described (25).

Calculations

Palmitate and glycerol kinetics—Plasma palmitate and glycerol rate of appearance (R_a) were calculated by dividing each tracer infusion rate by the average TTR obtained during the last 30 min of rest, exercise, and recovery periods. Palmitate rate of disappearance was assumed equal to palmitate R_a during rest. Steele's equation for non-steady-state conditions (41) was used to calculate kinetics during exercise and recovery periods. The effective volume of distributions used for palmitate (17) and glycerol (4) were 55 ml/kg FFM and 250 ml/kg BW respectively. Palmitate R_a provides an index of plasma FA availability during catecholamine-stimulated lipolysis, glycerol R_a provides a measure of FAs released primarily from lipolysis of adipose tissue triglycerides (TG) (25). Palmitate rate of disappearance provides an index of plasma FA tissue uptake. Glycerol R_a provides an index of whole body lipolytic rate and measures the rate at which glycerol is released into the systemic circulation, presumably from hydrolysis of adipose tissue and intramuscular and plasma TG.

Substrate oxidation—Total lipid and carbohydrate oxidation rates were calculated from rates of volume of oxygen consumed ($\dot{V}_{\text{O}_2}$) and $\dot{V}_{\text{CO}_2}$ as described by Frayn (9). Plasma palmitate oxidation rate was determined by dividing breath $^{13}\text{CO}_2$ production ($^{13}\text{CO}_2$ TTR $\dot{V}_{\text{CO}_2}$ production) by the plasma palmitate TTR. This value was corrected for $^{13}\text{CO}_2$ recovery as determined during the acetate infusion study (40). Plasma FFA oxidation was calculated by dividing palmitate oxidation rate by the proportional contribution of palmitate to total plasma FFA concentration. Nonplasma FA oxidation was determined by subtracting plasma FA oxidation from total FA oxidation (as determined by indirect calorimetry). We (18) assumed that intramuscular TG were the primary source of nonplasma FA oxidation and that plasma TG did not significantly contribute to oxidation during exercise.

Statistics

Pearson product moment correlations were used to evaluate relationships between descriptive and physiological variables. One-way ANOVA with Tukey honestly significant differences (HSD) post hoc tests were used to compare descriptive peak aerobic fitness and body composition parameters among groups. Two-way ANOVA (group $\times$ condition) with repeated measures and Tukey HSD were used to compare substrate kinetics and hormone concentrations among groups during each condition (i.e., rest, exercise, recovery). Confounding variables identified through Pearson product moment correlation were statistically controlled for by means of analyses of covariance. A P value ≤ 0.05 was considered statistically significant. All data are expressed as means $\pm$ SD unless specified otherwise.

RESULTS

Body Composition

There were no significant differences in body mass index, FFM, total body FM, limb and trunk FMs (DEXA), thigh and subcutaneous fat (MRI), and IMCL, extramyocellular lipid content, and liver fat content among groups (Table 1 and 2). However, HIV + RTV had significantly greater trunk fat-to-limb fat ratio (Table 2), greater trunk fat as a percentage of total FM (Control: $47 \pm 5\%$; HIV + no PI: $57 \pm 9\%$; HIV + RTV: $58 \pm 11\%$, $P < 0.05$), a

significantly greater percentage of VAT to TAT (VAT/TAT; Table 2), significantly lower limb fat as a percentage of total fat (Control: $53 \pm 5\%$; HIV + no PI: $43 \pm 9\%$; HIV + RTV: $43 \pm 11\%$, $P < 0.05$), and SAT/TAT than Control (SAT/TAT; Table 2). HIV + no PI had a trend toward greater trunk fat-to-total fat ($P < 0.07$) and significantly lower limb fat-to-total fat ratio ($P < 0.05$) compared with Control but were not different than HIV + RTV. HIV + no PI tended [$P < \text{not significant (NS)}$] to have greater VAT/TAT and lower SAT/TAT compared with Cntl (Table 2).

Exercise Capacity

$\dot{V}_{O_2 \text{ peak}}$ was not different among the three groups (Table 1); however, when HIV-positive subjects were combined into one group, $\dot{V}_{O_2 \text{ peak}}$ was 18% lower than Control ($P < 0.03$; data not shown). By design, the relative exercise intensity achieved and the absolute workload on the cycle ergometer during the tracer infusion study ($50\% \dot{V}_{O_2 \text{ peak}}$) were not different among the three groups (data not shown).

Fasting Metabolic Profile

At rest, there were no significant differences in glucose, insulin, C-peptide, FFA, epinephrine, norepinephrine levels, or energy expenditure between groups (Tables 3 and 4). However, when HIV-positive subjects were combined into one group, they tended to have higher insulin levels than Control ($P < 0.12$; data not shown). HIV + RTV but not HIV + no PI tended to have greater serum TG and lower HDL and LDL than Control (Table 3).

Hormone and Substrate Responses to Submaximal Exercise

In HIV + RTV, insulin levels were lower during exercise than at rest but did not change in the other groups (Table 4). Significant increases in epinephrine and norepinephrine levels occurred in all groups during exercise (Table 4). Despite exercising at the same relative intensity, blood lactate levels increased more in both HIV-positive groups than in Control (Table 4). FFA levels significantly increased from rest to submaximal exercise in Control, but they did not significantly increase in either HIV-infected group (Table 4). Furthermore, there was a trend toward lower FFA levels during submaximal exercise in HIV + RTV and HIV + no PI compared with Control ($P < 0.10$; Table 4). There were no significant differences in glucose, insulin, C-peptide, epinephrine, or norepinephrine between groups during exercise.

During recovery from exercise, epinephrine and norepinephrine returned to baseline levels in all groups (Table 4). Blood lactate also returned to baseline levels in both HIV-infected groups (Table 4). Insulin (Table 4) and C-peptide ($P < 0.06$ vs. HIV + no PI, $P < 0.001$ vs. Control; data not shown) during recovery were significantly greater in HIV + RTV than HIV + no PI and Control.

Lipid Kinetics

Glycerol R_a was not different among groups during rest or recovery. However, the increase in glycerol R_a during exercise was less in both HIV + RTV and HIV + no PI compared with Control (Fig. 1A). Palmitate R_a followed a similar trend to glycerol R_a ; it was similar among groups during rest and recovery but was significantly lower during exercise in HIV + RTV and HIV + no PI compared with Control (Fig. 1B).

During exercise, compared with rest, Control subjects increased palmitate, FFA, and total (plasma and nonplasma) FA oxidation rates ($\sim 50\%$), and these returned to resting levels after 60-min recovery (Fig. 1, C and D). The pattern was similar for both HIV-infected groups. However, the magnitude of the increase in palmitate and plasma FA oxidation rates

during exercise was less in the HIV-infected groups (Fig. 1, C and D). There were no differences in average percent palmitate uptake oxidized among groups during any condition (rest: Control $41 \pm 15\%$, HIV + no PI $41 \pm 10\%$, HIV + RTV $40 \pm 11\%$; exercise: Control $70 \pm 18\%$, HIV + no PI $65 \pm 13\%$, HIV + RTV $67 \pm 14\%$; recovery: Control $40 \pm 13\%$, HIV + no PI $36 \pm 17\%$, HIV + RTV $53 \pm 25\%$, $P = \text{NS}$). Overall, when $\dot{V}_{\text{O}_2 \text{ peak}}$ was used as a covariate (to correct for individual differences in fitness levels), the results were the same, indicating that subtle differences in fitness level or relative exercise intensity did not explain the failure of HIV-infected participants to increase lipolysis, palmitate, and plasma FA oxidation rates during exercise.

Correlation analyses indicated that lower lipolytic rates during exercise were not associated with lower limb or trunk fat measured by DEXA or lower SAT or VAT measured by MRI (data not shown). Glycerol R_a was significantly correlated with plasma FA oxidation ($r = 0.86$, $P < 0.001$), indicating a robust relationship between FA delivery and oxidation in all groups. There were no significant correlations between substrate kinetics and measures of insulin resistance/glucose tolerance during any condition.

DISCUSSION

The purpose of this study was to examine the effects of RTV-containing HAART on FA kinetics during rest, submaximal exercise, and recovery and to further examine the relationship among lipid kinetics, IMCL and hepatic lipid content, and insulin resistance/glucose tolerance in HIV-infected individuals. To our knowledge, this is the first study to quantify FA kinetics before, during, and after acute submaximal exercise in HIV-positive subjects taking HAART. The findings suggest that HIV infection and HAART, regardless of the presence or absence of RTV, was associated with a blunted lipolytic response and FA oxidation rate to moderate exercise compared with HIV-negative controls. This appeared to be primarily due to a slower rate of palmitate delivery during exercise, with a smaller contribution from reduced palmitate oxidation. Because all HIV-positive subjects were taking HAART, we were unable to determine whether the blunted lipolytic and FA oxidation during exercise was a result of HIV infection, HAART, or a combination of both.

We found lower lipolytic rates during moderate exercise ($50\% \dot{V}_{\text{O}_2 \text{ peak}}$) in both groups of HIV-positive-infected subjects taking HAART, regardless of RTV use, compared with Control. Similarly, plasma FFA concentration did not increase from resting values during exercise in HIV-infected groups, and plasma FFA concentration tended to be lower during exercise in both HIV-positive groups compared with Control. This was in contrast to our hypothesis, because we believed that lipolytic rate and FFA during moderate exercise in HIV-positive RTV would be elevated during exercise, as it is during rest in many HIV-positive subjects with lipodystrophy (36). It is unlikely that the lower lipolytic rates were due to a lower workload during exercise, because workload and $\dot{V}_{\text{O}_2}$ during exercise were similar between HIV-positive-infected and HIV-negative groups. There were also no significant correlations among $\dot{V}_{\text{O}_2 \text{ peak}}$, workload, or $\dot{V}_{\text{O}_2}$ during exercise and FA kinetics during rest, exercise, or recovery across groups. Moreover, the findings were the same when $\dot{V}_{\text{O}_2 \text{ peak}}$ was used as a statistical covariate, suggesting that the lower lipolytic rates during exercise were not primarily due to lower fitness level in HIV-positive subjects. Interestingly, plasma catecholamine levels during exercise and their change from resting levels were similar between HIV-positive and -negative groups. This suggests that HIV-positive subjects taking HAART, regardless of RTV use, had a blunted lipolytic response to moderate exercise-induced catecholamine levels. A blunted lipolytic response to exercise-induced catecholamines has been found in non-HIV-infected insulin-resistant states, including obesity (33) and diabetes (5). Previous studies in HIV-positive subjects have shown that the lipolytic response to catecholamines was either normal (14) or delayed (43). However,

during these studies, lipolysis was stimulated by exogenous administration of catecholamines rather than by exercise and consequently did not examine the ability of the sympathetic nervous system to augment lipolytic rate. Because a trend toward higher insulin levels was observed in HIV-positive subjects (when combined into one group), it is possible that elevated insulin levels played a role in the blunted lipolytic response to exercise, because insulin is a potent inhibitor of lipolysis (8). However, there was no relationship between insulin concentration and glycerol or palmitate R_a at rest or during exercise across groups. Therefore, it is unlikely that elevated insulin levels fully accounted for the blunted lipolytic response in HIV-positive subjects during exercise. Last, although not measured, decreased hormone-sensitive lipase activity may have played a role in the lower lipolytic response in HIV-positive subjects (46).

The lower rate of total FA oxidation during exercise in HIV-positive subjects taking HAART was a result of primarily lower plasma FA oxidation with a smaller contribution from nonplasma FA oxidation. The impairment in plasma FFA oxidation in HIV-positive subjects appeared to be due to impaired FFA delivery (lipolytic rate), as evidenced by the strong relationship between palmitate R_a and palmitate oxidation during exercise. Lower plasma FFA oxidation rate during moderate exercise was not related to lower limb fat or SAT in the present study. SAT, particularly upper body SAT, is the greatest contributor to lipolysis during rest and moderate exercise (~65%), with visceral fat contributing far less (~15%) in non-HIV-positive subjects (16). The lack of association between SAT, lipolytic rate, and plasma FA oxidation was likely due to heterogeneity in the distribution of adipose tissue among the HIV-positive subjects. HIV-positive subjects were selected for this study on the basis of taking RTV-containing HAART or taking HAART that did not include a PI. They were not selected on the basis of the presence or absence of fat redistribution (lipodystrophy). As a result, HIV-positive groups consisted of subjects with normal fat distribution, peripheral lipoatrophy only, visceral fat accumulation only, and a mixed phenotype (peripheral lipoatrophy with visceral accumulation). This heterogeneity may have affected our ability to detect group differences in lipid kinetics. Future studies elucidating the contributions of SAT and VAT to lipolytic rate and FFA oxidation and whether there is site-specific or overall insensitivity to exercise-induced catecholamines may provide insight into the mechanisms of fat redistribution in HIV-positive subjects taking HAART.

The degree to which exercise-stimulated palmitate oxidation was reduced in subjects with HIV was tightly related to exercise-stimulated lipolytic rate. This suggests that the reduced rate of lipid oxidation in skeletal muscle in HIV was largely due to a failure to mobilize FFA from adipose tissue stores rather than a primary defect in cellular FFA transport or mitochondrial FA utilization. Several other groups (21, 22, 31) have found reduced mitochondrial function/content in patients receiving HAART, and it is likely that we lacked statistical power to demonstrate subtle defects in nonplasma FFA oxidation in our HIV infected patients. We found a trend ($P < 0.07$) toward decreased nonplasma FA oxidation during submaximal exercise in HIV-positive subjects in addition to the reduction in plasma FFA oxidation. Nonplasma FA oxidation is a result of lipolysis, intracellular transport, and oxidation of intramuscular TG by the mitochondria (15, 32). It is unclear whether this reduction in nonplasma FA oxidation was due to impaired intramuscular lipolysis, FA transport (i.e., carnitine palmitoyltransferase I) into the mitochondria, or oxidative phosphorylation. Of note, when $\dot{V}O_{2\text{ peak}}$ was used as a covariate (data not shown), differences in nonplasma FA oxidation were no longer present. This may imply that the lower nonplasma FA oxidation was a result of a decreased quantity of skeletal muscle mitochondria and/or lower oxidative enzyme capacity from physical deconditioning (31). Further studies are warranted in this area.

Untreated HIV infection is associated with an elevated lipolytic rate and resting energy expenditure. HAART may further increase resting energy expenditure (19, 39). However, its effect on lipolytic rate remains unclear. On the basis of other studies (11, 30), we hypothesized that HIV-positive-infected subjects taking RTV-containing HAART would have elevated lipolytic rates during rest, moderate exercise, and recovery. However, we found similar resting and recovery lipolytic rates in both HIV-positive groups and HIV-seronegative controls. Both the dose and type of PI used may explain these findings. The metabolic side effects of PI are not a class effect; some PIs (e.g., indinavir) are more associated with insulin-resistant glucose metabolism, whereas others (e.g., atazanavir) appear to have a less toxic metabolic side effect profile. Indeed, we specifically chose LPV/RTV or RTV-boosted regimens (commonly prescribed regimens) as the study group for PI, because RTV has been associated with the development of lipid abnormalities, in particular hypertriglyceridemia, possibly due to its effect on lipolytic rate (1). HIV-positive subjects in the present study were taking RTV in the form of Kaletra (LPV 400 mg, RTV 100 mg BID, $n = 5$) or boosted RTV (100 mg qd, $n = 7$). Therefore, 58% of HIV-positive subjects taking RTV were only ingesting 100 mg qd, which may not have been an adequate dose to induce elevations in resting lipolytic rate. The short duration and lower dose may have limited the effects of RTV on lipolytic rate, although RTV administration has been shown to induce changes in serum FFA and TG in 4 wk in HIV-seronegative individuals (20). Last, duration of HAART was greater in HIV + no PI than HIV + RTV. This may have influenced the lack of differences in lipid kinetics and serum metabolic variables between these groups, since duration of HAART has been associated with an increased incidence of metabolic complications in HIV-positive subjects (10).

There were several limitations. This cross-sectional study was designed to explore the impact of RTV use on lipolysis and FA oxidation in stable HIV-positive patients receiving RTV-based HAART. As such, we are unable to determine a cause-and-effect relationship between the individual components of HAART and the observed metabolic defects. Clarification of a direct relationship between HAART use and metabolic side effects is challenging because patients with HIV infection require treatment with multiple antiretroviral agents, and these regimens are frequently changed because treatment fails, causing patients to be exposed to many different types of antiviral drugs. Because all HIV-positive subjects were taking HAART, it was not possible to determine whether HAART or HIV infection itself was related to impaired FA mobilization and oxidation. We did not quantify lipolytic rates in specific fat depots (SAT vs. VAT) during any condition and can thus only speculate on site-specific differences in exercise-stimulated lipolytic rate. The lack of statistical significance between HIV + RTV, HIV + no PI, and controls with respect to fasting insulin levels and hepatic lipid content may have been due to insufficient statistical power. The trends in these data suggest only a negative effect of RTV on insulin sensitivity and hepatic liver content. But these effects of RTV have been reported in other studies (26, 42, 45).

In summary, we found that patients with well-controlled HIV infection receiving HAART had a markedly blunted ability to oxidize FA during exercise at an intensity that corresponds to instrumental activities of daily living. These defects in FA oxidation were primarily due to decreased FFA delivery and a lesser contribution from impaired intramuscular TG oxidation. Further investigations into adipose tissue depot-specific responses to exercise-induced lipolysis and FA oxidation in HIV-positive subjects taking HAART are warranted.

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Fig 1.

A: glycerol. B: palmitate kinetics. C: palmitate oxidation rate during rest, submaximal exercise, and recovery. D: fatty acid (FA) oxidation rates [plasma (significance denoted above bar) and nonplasma (significance denoted mid-bar)] during exercise in the 3 groups. * $P < 0.01$; ** $P < 0.005$; \$ $P < 0.05$; \$\$ $P < 0.07$ vs. Control. R_a , rate of appearance; FFM, fat-free mass; FFA, free fatty acid; HIV, human immunodeficiency virus; PI, protease inhibitor; RTV, ritonavir.

Table 1

Descriptive characteristics

Variable	HIV Seronegative	HIV + No	PI HIV + RTV
<i>n</i>	10	10	12
Women/men	2/8	2/8	2/10
Age, yr	40±10	39±5	38±4
BMI, kg/m ²	25.7±3.4	24.7±4.2	27.1±5.7
Resting EE, kcal/min	1.1±0.2	1.2±0.1	1.2±0.2
Peak $\dot{V}O_2$, ml · kg ⁻¹ · min ⁻¹	33.1±9.7	27.7±6.0	26.4±5.4

Values are means ± SD. HIV + no PI, HIV-positive subjects taking antiretroviral medications but no protease inhibitor; HIV + RTV: HIV-seropositive subjects taking antiretroviral medications that include ritonavir; BMI, body mass index; EE, energy expenditure.

Table 2

Body composition parameters

Variable	HIV Seronegative	HIV + No PI	HIV + RTV
FFM, kg	53.4±8.9	56.7±12.3	56.1±8.5
Total fat mass, kg	16.6±8.0	17.2±14.9	16.3±10.3
Limb fat, kg	8.7±4.3	7.4±6.3	7.0±5.1
Trunk fat, kg	7.9±4.0	9.9±8.9	9.3±6.1
Trunk/limb ratio	0.9±0.2	1.4±0.6	1.6±0.8*
SAT, cm ²	174.2±94.4	177.4±170.6	161.6±114.9
VAT, cm ²	37.4±24.9	75.5±58.1	98.8±90.5
SAT/TAT, %	82±8	69±17	65±18*
VAT/TAT, %	18±8	31±17	35±18*
IMCL, %	2.9±2.7	2.5±2.3	2.3±1.3
EMCL, %	4.6±2.0	4.6±3.0	5.1±3.4
Liver lipid, %	1.3±0.9	1.6±1.0	2.2±1.6

Values are means ± SD. SAT, abdominal subcutaneous adipose tissue; VAT, visceral adipose tissue; TAT, total abdominal adipose tissue; IMCL, intramyocellular lipid; EMCL, extramyocellular lipid.

* $P < 0.05$.

Table 3

Serum metabolic parameters

Variable	HIV Seronegative	HIV + No PI	HIV + RTV
Triglycerides, mg/dl	95.5±50.5	145.1±62.2	311.1±338.0*
HDL cholesterol, mg/dl	57.1±20.2	45.2±15.8	40.7±13.1*
LDL cholesterol, mg/dl	113.2±18.0	90.6±28.3	78.7±29.8 [§]
Total cholesterol, mg/dl	188.2±34.0	164.8±36.9	166.8±45.4
Glucose, mg/dl			
0 min	86.2±4.2	90.3±7.3	88.3±3.6
2 hrs	106.7±26.7	133.2±33.5	115.0±22.4
Insulin, µU/ml			
0 min	4.3±2.2	6.4±6.9	7.9±4.7
2 hrs	31.0±23.5	36.7±38.7	50.9±39.1
C-peptide, ng/ml			
0 min	1.4±1.1	1.4±0.9	2.1±0.9

Values are means ± SD.

* $P < 0.07$;

[§] $P < 0.02$, all P values vs. HIV-seronegative subjects.

Table 4

Metabolic and endocrine parameters during rest, exercise, and recovery

Variable	HIV Seronegative	HIV + No PI	HIV + RTV
Insulin, $\mu\text{U/ml}$			
Rest	5.5 $\pm$ 3.7	7.2 $\pm$ 7.2	12.4 $\pm$ 9.3
70-min exercise	4.6 $\pm$ 2.3	4.8 $\pm$ 5.4	8.5 $\pm$ 5.2*
60-min recovery	3.8 $\pm$ 2.1	6.0 $\pm$ 6.8	9.6 $\pm$ 6.6 ^{\$}
Epinephrine, pM			
Rest	41.8 $\pm$ 27.1	34.8 $\pm$ 26.1	49.5 $\pm$ 50.5
70-min exercise	125.9 $\pm$ 58.6*	103.3 $\pm$ 49.6*	145.3 $\pm$ 142.1*
60-min recovery	45.2 $\pm$ 24.1**	33.8 $\pm$ 29.9**	62.5 $\pm$ 62.4**
Norepinephrine, nM			
Rest	167.9 $\pm$ 50.6	150.4 $\pm$ 62.6	234.2 $\pm$ 139.8
70-min exercise	737.6 $\pm$ 258.1*	578.4 $\pm$ 255.7*	747.1 $\pm$ 462.1*
60-min recovery	192.0 $\pm$ 52.2**	167.8 $\pm$ 68.1**	302.6 $\pm$ 167.4**
Lactate, $\mu\text{mol/l}$			
Rest	756.8 $\pm$ 405.9	916.3 $\pm$ 462.3	1,064.9 $\pm$ 510.7
70-min exercise	1,396.1 $\pm$ 824.2	946.4 $\pm$ 1141.8*	2,307.9 $\pm$ 1,337.6*
60-min recovery	659.1 $\pm$ 186.8	736.2 $\pm$ 466.8	1,028.1 $\pm$ 502.5
FFA, mM			
Rest	0.40 $\pm$ 0.24	0.31 $\pm$ 0.18	0.30 $\pm$ 0.19
70-min exercise	0.64 $\pm$ 0.53*	0.41 $\pm$ 0.24	0.42 $\pm$.20
60-min recovery	0.59 $\pm$ 0.33	0.42 $\pm$ 0.21	0.61 $\pm$ 0.28

Values are means $\pm$ SD. FFA, free fatty acid.* $P < 0.05$ rest $\rightarrow$ exercise;** $P < 0.05$ exercise $\rightarrow$ recovery;^{\$} $p < 0.02$ vs. HIV + no PI and Control.