

Alterations in lipid kinetics in men with HIV-dyslipidemia

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Abstract

Hypertriglyceridemia is common in individuals with HIV infection, but the mechanisms responsible for increased plasma triglyceride (TG) concentrations are not clear. We evaluated fatty acid and VLDL-TG kinetics during basal conditions and during a glucose infusion that resulted in typical postprandial plasma glucose and insulin concentrations in 6 men with HIV-dyslipidemia (body mass index [BMI]: 28 ± 2 kg/m²) and 6 healthy men (BMI: 26 ± 2 kg/m²). VLDL-TG secretion and palmitate rate of appearance (Ra) in plasma were measured by using stable-isotopically labeled tracer techniques. Basal palmitate Ra and VLDL-TG secretion rates were greater ($p < 0.01$ for both) in men with HIV-dyslipidemia (1.04 ± 0.07 $\mu\text{mol palmitate} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and 5.7 ± 0.6 $\mu\text{mol VLDL-TG} \cdot \text{L plasma}^{-1} \cdot \text{min}^{-1}$) than healthy men (0.67 ± 0.08 $\mu\text{mol palmitate} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and 3.0 ± 0.5 $\mu\text{mol VLDL-TG} \cdot \text{L plasma}^{-1} \cdot \text{min}^{-1}$). Basal VLDL-TG plasma clearance was lower in men with HIV-dyslipidemia (13 ± 1 mL·min⁻¹) than healthy men (19 ± 2 mL·min⁻¹) ($p < 0.05$). Glucose infusion decreased palmitate Ra (by ~50%) and VLDL-TG secretion rate (by ~30%) in both groups, but VLDL-TG secretion rate remained higher ($p < 0.05$) in subjects with HIV-dyslipidemia. These findings demonstrate that increased secretion of VLDL-TG and decreased plasma VLDL-TG clearance, during both fasting and fed conditions, contribute to hypertriglyceridemia in men with HIV-dyslipidemia. Although it is likely that increased FFA release from adipose tissue contributes to the increase in basal VLDL-TG concentration, other factors must be involved because insulin-induced suppression of lipolysis and systemic fatty acid availability did not normalize VLDL-TG secretion rate.

Keywords: stable isotopes, lipolysis, hypertriglyceridemia, lipoproteins, metabolism

Introduction

Dyslipidemia, characterized by high plasma triglyceride (TG) and low HDL-cholesterol concentrations(8, 36) , is commonly observed in patients with HIV infection and may be an important risk factor for coronary heart disease (3, 10, 23, 26). Although hypertriglyceridemia is often considered a side-effect of protease inhibitor (PI) therapy, and is found in up to 40% of PI-treated individuals with HIV-infection (5, 9), the initial description of hypertriglyceridemia in HIV-infected persons was reported years before the introduction of PIs and other combinations of highly active anti-retroviral therapy (HAART) (14). Therefore, it is likely that both HIV-infection itself and HIV-therapy are involved in the pathogenesis of HIV-dyslipidemia.

The physiological mechanisms responsible for hypertriglyceridemia in patients with HIV-infection are not completely clear, but must be a result of increased hepatic secretion of VLDL-TG into plasma, reduced TG clearance from plasma, or a combination of both. Plasma fatty acid availability is a major regulator of hepatic VLDL-TG production (27). Therefore, circulating insulin helps control whole-body TG flux during fed and fasted conditions by regulating lipolysis of adipose tissue TG (13) and plasma fatty acid availability for VLDL-TG production. A decrease in plasma insulin concentration during postabsorptive conditions increases lipolysis and plasma fatty acid availability, which increases VLDL-TG production. In contrast, the postprandial increase in plasma insulin concentration decreases lipolysis and fatty acid availability, which decreases endogenous VLDL-TG production and partially compensates for the delivery of dietary TG that are entering the systemic circulation as chylomicrons.

It is not clear whether fatty acid kinetics are altered in patients with HIV-dyslipidemia because of conflicting reports. Sekhar et al. (44) found the postabsorptive fatty acid rate of appearance (Ra) in plasma was higher in subjects with HIV-dyslipidemia than in normal controls, whereas Hadigan et al. (15) found the postabsorptive fatty acid Ra values were the same in patients with HIV-dyslipidemia and normal controls. The reason for the discrepancy between studies might be related to differences in insulin sensitivity in study subjects. Whole-body lipoprotein kinetics have not been carefully studied in patients with HIV infection. We are aware of one study that found an increased rate of VLDL-apolipoprotein-B100 (apo-B) secretion in patients with

HIV-dyslipidemia (42) but are not aware of any studies that evaluated VLDL-TG production rates.

In the present study we used stable-isotope tracer methods to evaluate fatty acid and VLDL-TG kinetics during basal conditions and during glucose infusion in men with HIV-dyslipidemia receiving HAART. All subjects had normal oral glucose tolerance to eliminate the confounding effect of severe insulin resistance on fatty acid metabolism. We hypothesized that HIV-dyslipidemia would be characterized by 1) increased fatty acid Ra and VLDL-TG secretion rates during postabsorptive conditions and 2) impaired suppression of fatty acid Ra and VLDL-TG secretion in response to hyperglycemia-hyperinsulinemia (achieved by glucose infusion).

Methods

Subjects

A total of 12 men (6 men with HIV-dyslipidemia; age: 44 ± 4 y and 6 healthy men, matched on BMI, age: 34 ± 5 y) participated in this study (**Table 1**). All subjects completed a comprehensive medical examination, which included a history and physical examination, an electrocardiogram, standard blood tests, and an oral glucose tolerance test. All subjects had normal fasting blood glucose concentrations and normal oral glucose tolerance; they had been weight-stable and sedentary (regular exercise <2 h/wk) for at least 3 months before the study. Subjects who consumed more than 3 alcoholic drinks per week were excluded from the study. Subjects with HIV-dyslipidemia were included if they had basal plasma TG concentration >250 mg/dL. Therefore, mean basal plasma TG concentration was greater in subjects with HIV-dyslipidemia than in healthy subjects (352 ± 24 mg/dL and 91 ± 14 mg/dL, $p<0.01$). All subjects with HIV infection had clinical evidence of a mixed lipodystrophy syndrome (16), determined by self-reported regional lipoatrophy (loss of facial or appendicular adipose tissue) and lipohypertrophy (accumulation of adipose tissue overlying the posterior cervical vertebrae, breasts, or trunk), which was confirmed by physical examination (16) and by an increased truncal:appendicular adipose tissue mass ratio assessed by DXA (51). The clinical characteristics of subjects with HIV-dyslipidemia, including duration of HIV infection and type of therapy, are shown in Table 2. No subject was taking other

medications known to affect lipid or glucose metabolism. All subjects with HIV-dyslipidemia had stable CD4 cell counts, and low or undetectable viral loads (<500 copies/mL) for at least 6 months before the study.

Written informed consent was obtained from all subjects before their participation in the study. The study was approved by the Human Studies Committee and the General Clinical Research Center (GCRC) Scientific Advisory Committee of Washington University School of Medicine in St. Louis, MO.

Experimental Protocol

Body composition analyses. Each subject's whole-body and regional fat masses (FM) and fat-free mass (FFM) were determined by dual-energy X-ray absorptiometry (DXA, Hologic QDR 44500/w, Waltham, MA) one week before the isotope infusion study. Trunk-to-appendicular fat mass ratio was calculated as trunk fat mass divided by the sum of the fat masses in the arms and legs as previously described (17, 32).

Isotope infusion study. Each subject completed an isotope infusion protocol to determine VLDL-TG kinetics on two separate occasions, in randomized order, approximately two weeks apart. Basal, postabsorptive fatty acid Ra and VLDL-TG secretion rate were measured after an overnight fast; on a second occasion fatty acid Ra and VLDL-TG secretion were measured during glucose infusion.

On the evening before the isotope infusion study, subjects were admitted to the inpatient unit of the GCRC at Washington University School of Medicine. At 1900 h, subjects consumed a standardized meal, containing a total of 12 kcal per kg body wt (55% of total energy from carbohydrates, 30% from fat, and 15% from protein). At 2000 h, subjects consumed a liquid formula (EnsureTM, Ross Laboratories, Columbus, OH), containing 250 kcal (40g carbohydrates, 6.1g fat, and 8.8g protein) to ensure complete filling of hepatic glycogen stores, and then fasted until the completion of the study. For the glucose infusion study, an infusion of dextrose ($5.5 \text{ mg}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$) was initiated at 2100 h and maintained until the completion of the isotope infusion study the next day.

The following morning, at 0530 h, a catheter was inserted into an antecubital vein to administer stable isotope labeled tracers, and a second catheter was inserted into a hand vein of the opposite arm; the hand was heated to 55°C using a thermostatically

controlled box to obtain arterialized blood samples (24). At 0700 h, a bolus of [1,1,2,3,3-²H₅]glycerol (75 μmol/kg body wt), dissolved in 0.9% NaCl, was administered through the catheter in the antecubital vein, and a constant infusion of [2,2-²H₂]palmitate (0.03 μmol·kg body wt⁻¹·min⁻¹), bound to human albumin (Centeon LLC, Kankakee, IL), was started and maintained for 4 h. At the completion of the study, all catheters were removed. All isotope labeled tracers were obtained from Cambridge Isotope Laboratories, Andover, MA.

Blood sampling. Blood samples were obtained immediately before the administration of stable-isotope labeled tracers to determine plasma substrate (fatty acid, glucose and TG), and insulin concentrations, and background palmitate tracer-to-tracee ratio (TTR) in plasma, and glycerol TTR in VLDL-TG. Blood samples were obtained again at 5, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600, 660, and 720 minutes after the start of the isotope infusion to determine palmitate TTR in plasma, and glycerol TTR in VLDL-TG. Blood samples to determine plasma glucose and VLDL-TG concentrations were obtained every 2 h during the entire study period, and blood samples for the determination of plasma insulin concentrations were collected at 4, 8, and 12 h. Subjects remained in bed for the entire duration of the study.

Sample collection. Blood samples to determine fatty acid and TG concentrations and TTRs were collected in chilled tubes containing EDTA; samples to determine plasma insulin concentrations were collected in chilled tubes containing EDTA and trasylol. Immediately after collection, blood samples were placed in ice and plasma was separated by centrifugation within 30 min of collection. Aliquots of plasma (2 mL) were kept in the refrigerator for subsequent isolation of VLDL. The remaining plasma samples were stored at -70°C until final analyses were performed. Blood samples to determine plasma glucose concentration were collected in tubes containing heparin and analyzed immediately after collection.

Isolation of VLDL. Immediately after completion of the isotope infusion study, the VLDL fraction in plasma was isolated by ultracentrifugation as previously described (11). Briefly, 1.5 mL of each plasma sample was transferred into Optiseal tubes (Beckman Instruments Inc., Palo Alto, CA), overlaid with a saline solution (density = 1.006 kg/L) and centrifuged for 16 h at 45,000 rpm and 10°C in a 50.4 Ti rotor (Beckman

Instruments Inc., Palo Alto, CA). The top layer, containing VLDL, was removed by tube slicing (Beckman Instruments Inc., Palo Alto, CA). The exact volumes that were recovered (~1.3 mL) were recorded. Isolated VLDL fractions were stored at -70°C until final analyses were performed.

Sample analyses. Plasma glucose concentration was determined by using an automated glucose analyzer (Yellow Spring Instruments Co, Yellow Springs, OH). Plasma insulin concentration was measured by using radioimmunoassay (19). VLDL-TG concentration was measured enzymatically (SIGMA Chemicals Inc., St. Louis, MO). Plasma fatty acid concentrations were quantified by using gas chromatography (Hewlett-Packard 5890-II, Palo Alto, CA) after adding heptadecanoic acid to plasma as an internal standard (35).

All isotopic enrichments were measured by selected ion monitoring (SIM) electron impact (EI) ionization gas-chromatography/mass-spectrometry (GC/MS) using an MSD 5973 system (Hewlett-Packard, Palo Alto, CA). TTRs were determined as previously described (34, 35). Instrument response was calibrated by using standards of known isotopic enrichment.

Calculations

Palmitate Ra in plasma was calculated by dividing the palmitate tracer infusion rate by the average arterial palmitate TTR obtained between 1.5 and 4 h (Steele's equation for steady state conditions (46, 50)). Due to technical difficulties, palmitate Ra could not be determined for one healthy subject.

The fractional catabolic rate (FCR) of VLDL-TG, which represents the fraction of the VLDL-TG pool that disappears from plasma per unit of time, was determined by mono-exponential slope analysis as previously described (34), and expressed as $\text{pools}\cdot\text{hr}^{-1}$. In all subjects, the VLDL-TG FCR was $\leq 0.4 \text{ pools}\cdot\text{h}^{-1}$, and we have previously shown that the mono-exponential slope analysis provides accurate FCR values for VLDL-TG pools with a turnover rate in this range (34). It was assumed that the rate of VLDL-TG secretion was equal to the rate of VLDL-TG catabolism, because plasma VLDL-TG concentration remained constant throughout the entire 12-h study period (data not shown). The absolute rate of VLDL-TG secretion (equal to the absolute rate of VLDL-

TG catabolism) was calculated as: 1) total VLDL-TG secretion rate, which represents the amount of VLDL-TG produced by the liver and secreted into the bloodstream, and 2) VLDL-TG secretion rate per unit of plasma as follows:

$$\text{Total VLDL-TG secretion rate (in } \mu\text{mol}\cdot\text{min}^{-1}\text{)} = (\text{VLDL-TG FCR} \times C_{\text{VLDL-TG}} \times \text{PV})/60$$

$$\text{VLDL-TG secretion (in } \mu\text{mol L plasma}^{-1}\cdot\text{min}^{-1}\text{)} = (\text{VLDL-TG FCR} \times C_{\text{VLDL-TG}})/60$$

where $C_{\text{VLDL-TG}}$ is the concentration of VLDL-TG in plasma, and PV is the plasma volume, which was estimated based on each subject's FFM ($\text{PV} = 0.055 \text{ L} \times \text{kg FFM}^{-1}$ (7, 12)). It was assumed that PV was equal to the VLDL-TG volume of distribution, because VLDL is restricted to the plasma compartment and does not enter the interstitial space or the lymphatic system (39).

The plasma clearance of VLDL-TG (in $\text{mL}\cdot\text{min}^{-1}$) was calculated by dividing the rate of VLDL-TG disappearance (at steady state equal to secretion rate; in $\mu\text{mol}\cdot\text{min}^{-1}$) by the VLDL-TG concentration in plasma (in μM).

Statistical Analysis

A Student's t-test for independent samples was used to evaluate the significance of differences in age and body composition between men with HIV-dyslipidemia and healthy men. A two-way (subject x treatment) analysis of variance (ANOVA) with repeated measures on the last factor was used to assess the significance of differences in plasma substrate and insulin concentrations and palmitate and VLDL-TG kinetics between the two groups during basal conditions and glucose infusion. A p-value of ≤ 0.05 was considered statistically significant. All data are expressed as means \pm SEM.

Results

Plasma substrate and insulin concentrations

Basal conditions. Basal plasma glucose and insulin concentrations were not significantly different between men with HIV-dyslipidemia and healthy men. Basal plasma FFA and VLDL-TG concentrations were significantly higher ($p < 0.05$) in men with HIV-dyslipidemia than in healthy men (**Table 3**).

Glucose infusion. The total amount of glucose that was infused during the study was 490 ± 21 g in healthy men and 474 ± 17 g in men with HIV-dyslipidemia ($p=NS$). Glucose infusion raised plasma glucose concentrations by ~45% and plasma insulin concentration by ~400% and decreased total plasma FFA concentration by ~65% both in men with HIV-dyslipidemia and healthy men (treatment effect: $p<0.01$ for all comparisons; no difference between groups). Glucose infusion had no effect on plasma VLDL-TG concentration. (**Table 3**).

Fatty acid kinetics

Basal palmitate Ra was ~60% greater ($p<0.01$) in men with HIV-dyslipidemia than healthy men. Glucose infusion caused a marked reduction in palmitate Ra in both groups ($p<0.01$); however, the relative response to glucose infusion was greater in men with HIV-dyslipidemia than in healthy men ($p<0.02$ for treatment x group interaction). Similarly, the absolute decline in palmitate Ra was greater ($p<0.05$) in men with HIV-dyslipidemia ($0.64 \pm 0.07 \mu\text{mol}\cdot\text{kg body wt}^{-1}\cdot\text{min}^{-1}$) than healthy men ($0.34 \pm 0.08 \mu\text{mol}\cdot\text{kg body wt}^{-1}\cdot\text{min}^{-1}$). Therefore, absolute palmitateRa was similar in the two groups during glucose infusion. (**Figure 1**).

VLDL-TG kinetics

Basal VLDL-TG FCR was ~30% lower in men with HIV-dyslipidemia ($0.23 \pm 0.02 \text{ pools}\cdot\text{h}^{-1}$) than in healthy men ($0.33 \pm 0.02 \text{ pools}\cdot\text{h}^{-1}$); glucose infusion decreased VLDL-TG FCR to $0.26 \pm 0.04 \text{ pools}\cdot\text{h}^{-1}$ in healthy men and to $0.15 \pm 0.02 \text{ pools}\cdot\text{h}^{-1}$ in men with HIV-dyslipidemia (group effect: $p<0.05$; treatment effect: $p<0.01$; no treatment x group interaction).

During basal conditions, the VLDL-TG secretion rate was twice as high in men with HIV-dyslipidemia than in healthy men (**Figure 2**). Glucose infusion caused a ~30% reduction ($p<0.01$) in VLDL-TG secretion rate in both men with HIV-dyslipidemia and healthy men, but the rate of VLDL-TG secretion remained higher ($p<0.01$) in men with HIV-dyslipidemia than healthy men during glucose infusion.

Basal VLDL-TG plasma clearance was ~30% lower in men with HIV-dyslipidemia than in healthy men (13 ± 1 and $19 \pm 2 \text{ mL}\cdot\text{min}^{-1}$); glucose infusion

significantly decreased VLDL-TG plasma clearance in both men with HIV-dyslipidemia (to $9 \pm 1 \text{ mL}\cdot\text{min}^{-1}$) and healthy men (to $15 \pm 1 \text{ mL}\cdot\text{min}^{-1}$) (group effect: $p < 0.05$; treatment effect: $p < 0.05$; no treatment x group interaction).

Discussion

Individuals with HIV infection, particularly those given HAART, are at increased risk of developing HIV-dyslipidemia. In this study, we evaluated some of the potential physiological mechanisms responsible for increased plasma TG concentrations by using stable isotope tracer methods to determine fatty acid and VLDL-TG kinetics during post-absorptive and “feeding” (i.e., glucose infusion) conditions in men with HIV-dyslipidemia. Subjects with abnormal glucose tolerance or diabetes were excluded to minimize the potential confounding effect of severe insulin resistance on lipid kinetics. Our data demonstrate that basal fatty acid Ra and VLDL-TG secretion rates are greater in men with HIV-dyslipidemia than in healthy men. Glucose infusion and concomitant hyperinsulinemia suppressed fatty acid Ra and VLDL-TG secretion rate in both healthy men and in those with HIV-dyslipidemia, but VLDL-TG secretion remained elevated in subjects with HIV-dyslipidemia during glucose infusion. These findings suggest that increased rates of VLDL-TG secretion during both fed and fasted conditions contribute to hypertriglyceridemia associated with HIV-infection. It is likely that the increased basal rate of fatty acid release into plasma stimulated the increase in basal VLDL-TG secretion in men with HIV-dyslipidemia, but other factors must also be involved because suppression of lipolysis and systemic plasma FFA availability by hyperinsulinemia did not normalize VLDL-TG secretion rate.

Basal plasma FFA concentrations and palmitate Ra were much greater in men with HIV-dyslipidemia than in healthy men. HIV infection itself rather than HAART was probably responsible for the alterations in fatty acid metabolism because basal glycerol kinetics, an index of adipose lipolytic activity, have been reported to be similar in subjects with HIV-dyslipidemia and lipodystrophy either receiving HAART or not (49). The mechanism responsible for the increase in lipolytic rate in our subjects is not known. Even though insulin is a major regulator of basal lipolytic rate (21), and adipose tissue insulin resistance has been demonstrated in a previous study conducted in patients

with HIV-lipodystrophy (48), it is unlikely that insulin resistance was responsible for increased lipolysis in our subjects. Glucose infusion and concomitant hyperinsulinemia caused a similar relative decrease and a greater absolute decrease in palmitate Ra in our men with HIV-dyslipidemia than in healthy men. Therefore, our data suggest that the sensitivity of adipose tissue lipolysis to insulin is not impaired in men with HIV-dyslipidemia who have normal oral glucose tolerance.

It is possible that the greater basal lipolytic rate in our men with HIV-dyslipidemia was due to increased β -adrenergic stimulation of adipose tissue. Other investigators have found increased plasma and urinary catecholamine concentrations in subjects with HIV-infection (40, 49). In addition, increased β -adrenergic stimulation is responsible for increased basal lipolytic rates in other inflammatory diseases, such as cancer (25).

Our HIV-infected subjects had several important risk factors for dyslipidemia. The presence of regional lipodystrophy is strongly associated with hypertriglyceridemia (16). All our subjects had a mixed lipodystrophy syndrome, which was characterized by the presence of lipoatrophy of the face and arms and lipohypertrophy of the posterior cervical vertebrae and abdomen. In addition, treatment with PIs increases the risk of developing hypertriglyceridemia (5, 9). Four of our six HIV-infected subjects were receiving PI therapy at the time of our study; the other two subjects were not actively being treated with PIs but had received PI therapy for 3-5 years in the past. Although improvement of dyslipidemia has been reported after switching from PIs to non-nucleoside reverse transcriptase inhibitors (30), no improvement occurred in body fat redistribution or blood lipids in our two subjects who stopped PI therapy approximately 2 years before our study. Despite the heterogeneity in medications, fatty acid and lipoprotein kinetics were similar in all our subjects with HIV-dyslipidemia.

Although our men with HIV-dyslipidemia may have had normal insulin sensitivity in peripheral adipose tissue, the high rates of fatty acid release into plasma could cause resistance to insulin action in other tissues, such as the liver or skeletal muscles (6). In fact, it has recently been shown that administration of acipimox, which inhibits adipose tissue lipolysis, improved insulin sensitivity in men with HIV-infection and fat redistribution (18).

Hepatic fatty acid availability is the major regulator of VLDL-TG secretion (27). Therefore, increased release of fatty acids from subcutaneous adipose tissue probably contributed to the elevated VLDL-TG secretion rate in our men with HIV dyslipidemia. Fatty acid oxidation was found not to be increased in subjects with HIV-dyslipidemia and lipodystrophy (44), which suggested that excess fatty acids that are delivered to the liver are re-esterified and stored as intra-hepatic TG and/or secreted as part of VLDL. It is also likely that increased fatty acids from other sources also contributed to VLDL-TG production in our men with HIV-dyslipidemia. Although we did not measure intra-hepatic fat content, it has previously been found that intra-hepatic TG content is greater in HIV-infected subjects with dyslipidemia and lipodystrophy than in healthy subjects (47). Our men with HIV-dyslipidemia had ~40% more trunk fat mass compared with the healthy; most of the excess trunk fat was probably located within the intra-abdominal region (40). Intra-peritoneal fat cells are more lipolytically active than adipocytes in subcutaneous adipose tissue (2, 41) and the inhibitory effect of insulin on lipolysis is less pronounced in intra-abdominal than subcutaneous adipose tissue (31). Fatty acids derived from *de novo* lipogenesis contribute <5% to total VLDL-TG secretion in healthy men (1). However, increased rates of hepatic *de novo* lipogenesis have been observed in patients with HIV infection (20). Therefore, it is likely that increased hepatic fatty acid availability, derived from lipolysis of subcutaneous, intra-abdominal, and intrahepatic TG, and from *de novo* lipogenesis, may have contributed to the high rates of basal VLDL-TG secretion in our men with HIV-dyslipidemia.

Although we did not measure the rate of VLDL-apo-B secretion in the current study, our data are consistent with those from a previous study that found significantly higher rates of VLDL-apoB secretion in HIV-infected subjects receiving HAART than in a healthy control group (42). The increased rate of VLDL-apoB secretion was predominantly due to the secretion of large, buoyant, TG-rich VLDL particles (VLDL₁) and was accompanied by a reduction in transfer of lipid from VLDL₁ to denser, less TG-rich VLDL₂ (42). Liang et. al. (29) found that PIs inhibit proteosomal degradation of apoB in hepatoma cells, which may be an important mechanism for the increase in VLDL-apoB production observed *in vivo*. Taken together, our findings and those from these previous studies (42) suggest that both the number and the size of VLDL particles

secreted by the liver are greater in subjects with HIV-dyslipidemia than in healthy subjects.

The effect of glucose and/or insulin on VLDL-TG metabolism in subjects with HIV-infection has not been studied previously. Hyperglycemia-hyperinsulinemia (1) and conditions that favor hepatic lipogenesis (22, 43), increases the relative contribution of *de novo* synthesized fatty acids to total VLDL-TG secretion. Furthermore, hyperglycemia-hyperinsulinemia inhibits fatty acid oxidation in the liver (45), which increases the availability of fatty acids delivered to the liver for VLDL-TG synthesis. However, the effect of insulin on adipose tissue lipolysis markedly reduces the delivery of plasma fatty acids to the liver and total hepatic fatty acid availability, which probably mediates a decrease in VLDL-TG production during hyperglycemia-hyperinsulinemia (28, 33). We found that the relative decrease of VLDL-TG secretion in response to glucose infusion was not different in men with HIV-dyslipidemia and healthy men. Nonetheless, the absolute rate of VLDL-TG secretion was greater in men with HIV-dyslipidemia than in healthy men during glucose infusion despite similar rates of fatty acid Ra and plasma fatty acid concentrations, suggesting that factors other than plasma fatty acid availability stimulate VLDL-TG production in men with HIV-infection.

Plasma clearance of VLDL-TG was lower in men with HIV dyslipidemia than in healthy men during both basal conditions and glucose infusion. The reduced rate of clearance may be related to alterations in lipoprotein lipase (LPL) activity associated with HIV-infection or HIV-therapy. Baril et. al. (4) found that post-heparin LPL activity was ~50% lower in men with HIV-dyslipidemia receiving HAART than in age-matched healthy men and Sekhar et al. (44) reported impaired disposal of chylomicrons and VLDL-TG in patients with HIV-lipodystrophy during postprandial conditions. In addition, two weeks of treatment with ritonavir decreased hepatic lipase activity in healthy men (37), and both saquinavir and indinavir reduced LPL activity *in vitro* in cultured adipocytes (38). Therefore, these data suggest that medications used to treat HIV infection affect whole-body LPL activity, but it is not known if this effect is specific to certain tissues.

In summary, the results of this study demonstrate the presence of considerable abnormalities in fatty acid and lipoprotein metabolism in men with HIV-dyslipidemia.

Moreover, these alterations in lipid metabolism occurred before there was clinical evidence of insulin-resistant glucose metabolism, such as impaired oral glucose tolerance or type 2 diabetes. The rate of hepatic VLDL-TG secretion during fed and fasted conditions was much greater, and clearance of VLDL-TG from plasma was slightly lower, in men with HIV-dyslipidemia than in healthy men. Therefore, both increased VLDL-TG secretion into plasma and decreased plasma VLDL-TG clearance contribute to hypertriglyceridemia. Although it is likely that increased basal rates of fatty acid release into plasma stimulated basal VLDL-TG production in men with HIV-dyslipidemia, other factors must be involved because suppression of lipolysis by hyperinsulinemia did not normalize VLDL-TG secretion rate.

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Figure Legends

Figure 1. Palmitate Ra in the basal, postabsorptive state and during glucose infusion (5.5 mg·kg FFM⁻¹·min⁻¹) in healthy men and men with HIV-dyslipidemia. * Value significantly different from corresponding value in healthy men, p<0.01. † Value significantly different from corresponding value during basal conditions, p<0.01. Values are means ± SEM. Data for healthy men represent the average value for 5 of the 6 subjects due to technical difficulties in the sample analysis of one subject.

Figure 2. Rate of VLDL-TG secretion into plasma in the basal, postabsorptive state and during glucose infusion (5.5 mg·kg FFM⁻¹·min⁻¹) in healthy men and men with HIV-dyslipidemia. * Value significantly different from corresponding value in healthy men, p<0.01. † Value significantly different from corresponding value during basal conditions, p<0.05. Values are means ± SEM.

Table 1. Body composition of study subjects.

	Healthy Men	Men with HIV- dyslipidemia
Body weight (kg)	83 ± 3	83 ± 4
BMI (kg/m ²)	26 ± 2	28 ± 2
Fat mass (kg)	15 ± 1	18 ± 3
Fat mass (% total body weight)	19 ± 1	21 ± 3
Fat-free mass (kg)	67 ± 3	65 ± 2
Trunk:appendicular fat mass ratio	1.1 ± 0.1	1.9 ± 0.2 *

Values are means ± SEM. * Value significantly different from corresponding value in healthy men; p<0.05.

Table 2. Clinical characteristics of subjects with HIV-dyslipidemia.

Subject	Duration of HIV infection (Yrs)	Current medication	Duration of PI exposure (Yrs)	CD4 count (cells/μL)
1	6	NFV, 3TC, D4T	3.7	869
2	6	NVP, AZT, 3TC	3.0	778
3	8	IDV, AZT, 3TC	3.3	1027
4	2	NFV, AZT, 3TC	1.7	1154
5	9	ABC, DDI, EFV	5.0	658
6	9	NFV, AZT, 3TC	5.2	129
Mean	7 \pm 1		3.7 \pm 0.5	769 \pm 146

3TC=lamivudine, ABC=abacavir, AZT=zidovudine, D4T=stavudine, DDI=didanosine, EFV=efavirenz, IDV=indinavir, NFV=nelfinavir, NVP= nevirapine. Values are means \pm SEM.

Table 3. Plasma substrate and insulin concentrations.

	Healthy men		Men with HIV-dyslipidemia	
	Basal	Glucose	Basal	Glucose
Glucose (mM)	4.9 ± 0.1	7.1 ± 0.2 [†]	4.8 ± 0.1	7.2 ± 0.3 [†]
Insulin (μU/mL)	5 ± 1	24 ± 6 [†]	8 ± 2	37 ± 5 [†]
FFA (mM)	0.31 ± 0.04	0.10 ± 0.03 [†]	0.46 ± 0.05*	0.13 ± 0.02 [†]
VLDL-TG (mM)	0.6 ± 0.1	0.5 ± 0.1	1.6 ± 0.2*	1.7 ± 0.2*

Values are means ± SEM. Glucose and insulin: average values obtained at baseline, before the isotope infusion; FFA: average values during the first 30 min of the isotope infusion, VLDL-TG: average values during the entire isotope infusion study (12 h). * Value significantly different from corresponding value in healthy men (p<0.05). [†] Value significantly different from corresponding value during basal conditions. (p<0.05).

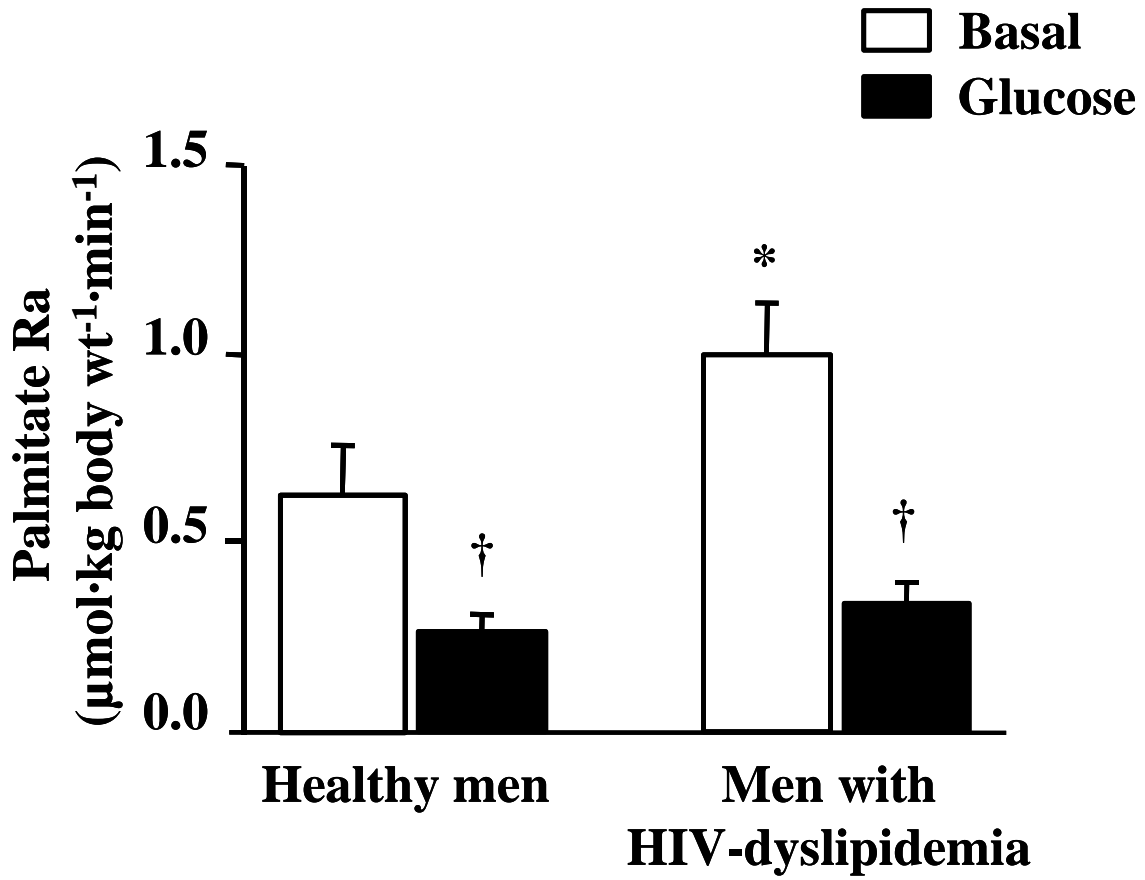


Figure 1

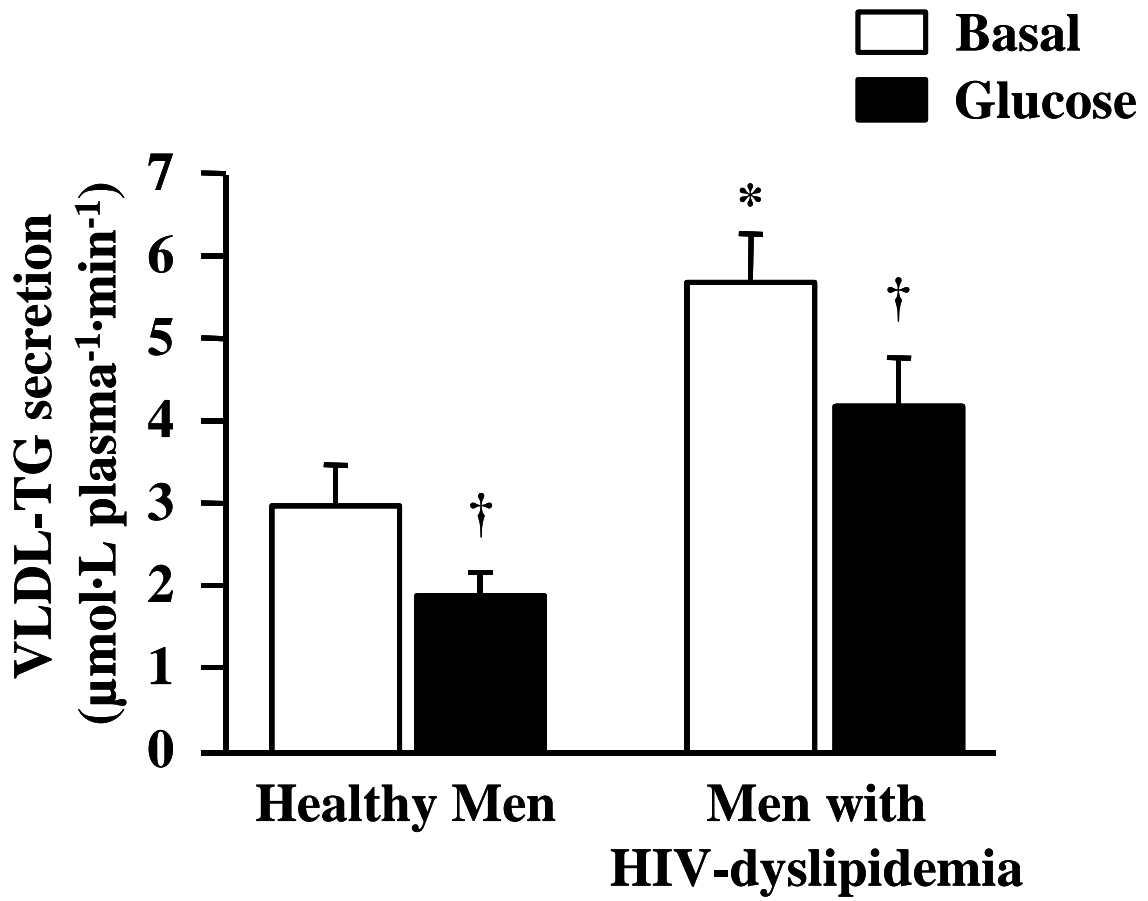


Figure 2