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Metabolic complications of antiretroviral therapy

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Publication date
2003

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Citation for published version (APA):
van der Valk, M. (2003). *Metabolic complications of antiretroviral therapy*. [Thesis, fully internal, Universiteit van Amsterdam].

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Highly active antiretroviral therapy-induced lipodystrophy has minor effects on HIV induced changes in lipolysis but normalises resting energy expenditure

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The Journal of Clinical Endocrinology & Metabolism 2002, **87**(11):5066-5071

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Introduction

Combination antiretroviral therapy, including protease inhibitors, for the treatment of HIV-1 infected patients has been associated with the development of a fat redistribution syndrome (lipodystrophy), which may include both central fat accumulation and peripheral fat wasting[1]. In patients with lipodystrophy severe insulin resistance has been documented, which is associated with marked increases in plasma insulin concentrations. Insulin is the main inhibitor of lipolysis. In patients with and without lipodystrophy using highly active antiretroviral therapy (HAART) the postabsorptive concentration of free fatty acids (FFA) is higher and the suppression of FFA concentrations by insulin is inhibited.[2-4]

The natural course of HIV-1 infection is associated with both an increase in whole body lipolysis and an increase in resting energy expenditure (REE). [5-8] The cause of these changes is unknown. Catecholamines are the main stimulators of both metabolic processes, but are not increased in HIV-1 infected patients without concomitant opportunistic infection. Moreover, the lipolytic response to the administration of epinephrine is normal in such patients.[7] It is unknown if lipolysis is regulated differently and whether the REE is increased in HIV-1 infected subjects with lipodystrophy compared to patients without lipodystrophy. To answer these questions, we evaluated basal REE and lipolysis and the sensitivity of both metabolic processes to an epinephrine infusion, resulting in slightly increased, but still physiological epinephrine plasma levels, in HIV-infected patients with the lipodystrophy syndrome. We compared these with data obtained in untreated HIV-1 infected patients without concomitant opportunistic infections.

Subjects and Methods

Subjects

We studied nine HIV-1 positive men with lipodystrophy (LD) who were included in the 'Reverse' study. This is an ongoing protocol in which HIV-1 infected patients with lipodystrophy are examined for reversibility of the syndrome when replacing the protease inhibitor component in their regimen by the nucleoside reverse transcriptase inhibitor abacavir. Patients eligible for inclusion in this study have to use a protease inhibitor-containing regimen and have a plasma HIV-1 RNA level below 400 copies per milliliter for at least 6 months. Patients with diabetes melllitus, defined by a postabsorptive plasma glucose concentration above 7.0 mmol/l, were excluded[9].

After 6 weeks of adding abacavir (300mg, two times daily) to their current regimen, patients were randomized to either discontinue their protease inhibitors immediately, or continue protease inhibitor use for another 12 weeks and then stop. Patients referred for this protocol had lipodystrophy in the opinion of their treating physician. Prior inclusion this was confirmed by physical examination and by obtaining the patients' history by two study physicians. Lipodystrophy was defined as the presence of peripheral lipoatrophy, central fat accumulation or both. All assessments, reported for the LD group, were performed six weeks after adding abacavir to the current antiretroviral regimen, but prior to withdrawal of protease inhibitors. We included patients who reached this point between February 2000 and April 2001. In the final analysis, one of the nine patients was excluded, because he had developed a left bundle branch block on EKG, a contraindication for the administration of epinephrine.

We compared the results with those obtained in our hospital in five HIV-infected patients who were not treated with HAART (HIV). All subjects from the HIV group were weight-stable and did not have any

active opportunistic disease. Patients who had fever (temperature > 37.5 °C), diarrhoea, renal, hepatic, or endocrine disease, malignancies other than Kaposi's sarcoma of the skin, weight loss or clinically active opportunistic infection in the two months prior to study entry were excluded. Two out of five patients used zidovudine monotherapy, while the rest were completely therapy naïve. The results from this control group have been published previously.[7]

Both studies were approved by the institutional review board of the Academic Medical Center in Amsterdam. Written informed consent was obtained from all subjects.

Study design

The subjects were admitted to the metabolic clinical research center and studied in the supine position. Following a 12 hour fast, a catheter was inserted antegrade in a deep antecubital vein of each arm. One catheter was used for sampling of arterialized blood using a heated handbox (60 °C). The other catheter was used for infusion of [$^2H_5$]-glycerol (1,6 μmol/l priming dose and 0.11 μmol/ kg/min), and epinephrine (15 ng/kg/min). After blood samples were taken for determination of background isotope enrichment, intravenous infusion of of [$^2H_5$]-glycerol was started and continued for 120 minutes. Epinephrine (15 ng/kg/min) was infused during the last 60 minutes of isotope infusion. This specific dose of epinephrine was chosen because it results in adequate stimulation of lipolysis at physiological plasma concentrations of epinephrine. Blood samples were obtained at 45, 50, 55 and 60 minutes of isotope infusion to determine basal lipid kinetics and every 5 minutes during epinephrine infusion to determine the lipolytic response to epinephrine. Blood samples for hormone concentrations were obtained every 15 minutes during epinephrine infusion. Blood pressure and heart rate were monitored every 10 minutes.

All samples were put on ice immediately. Plasma was separated by centrifugation at 4 °C within 10 minutes and stored at -20 °C.

Analytical procedures

Samples for catecholamine analysis were collected in 5 ml glass tubes containing reduced gluthathione and ethyleneglycol-bis-(2-aminoethyl) tetra acetic acid. Plasma epinephrine and norepinephrine concentrations were determined by HPLC (high performance liquid chromatography) and electrochemical detection in controls (HIV) and fluorescence detection in the LD group[10]. Plasma insulin concentration was determined by a radioimmuno-assay (Insulin RIA 100, Pharmacia Diagnostic AB, Uppsala, Sweden, intra-assay coefficient of variation (c.v.): 3-5 %, inter-assay c.v.: 6-9 %, detection limit: 15 pmol/L).

Blood for analysis of glycerol enrichment was collected in pre-chilled heparinized tubes. Isotope enrichment of glycerol in plasma was determined by gas chromatography–mass spectrometry using an MSD 5971 system in the HIV group (Hewlett Packard, Palo Alto, CA, USA) [11] and in the LD group as described previously[12].

Body composition

Body composition was measured with a body impedance analyzer (BIA 109 Akern, Florence, Italy) the morning before the start of the isotope infusion study.

Indirect calorimetry

Oxygen consumption (VO_2) and CO_2 production (VCO_2) were measured by indirect calorimetry using a ventilated hood system (Sensormedics model 2900, Anaheim, Ca). VO_2 and VCO_2 were measured continuously during the first 30 minutes of glycerol infusion without epinephrine

infusion and during the last 30 minutes of epinephrine infusion. Resting energy expenditure was calculated using formulas for substrate oxidation as proposed by Frayn.[13]

Calculations

Steele's equation for steady state conditions as adapted for the use of stable isotopes [14] was used to calculate baseline glycerol Rate of appearance (Ra). During epinephrine infusion, glycerol Ra was calculated using the Steele equation for non-steady state kinetics. The effective volume of distribution of glycerol was assumed to be 235 ml/kg. Enrichment and concentration data obtained during epinephrine infusion were smoothed by spline fitting[15] and substrate kinetics were calculated using these smoothed data.

Statistical analysis

The Ra glycerol data derived after spline fitting were analysed using the SAS proc mixed procedure (SAS Institute Inc, Version 8, Cary, North Carolina), which accommodates repeated measurements. The 'first level auto-regressive' covariate structure appeared to be the most appropriate after comparing several covariate structures through restricted maximum likelihood calculations. In all initial analyses the explanatory variables were group, time (categorical) and their interaction. For Ra glycerol an additional model was used to correct the outcome measurements for possible baseline differences. When medians between the two groups were compared the Kruskall Wallis method was used. The resting energy expenditure results were compared between the two groups using a linear regression model adjusted for the amount of lean body mass in kg. The overall level of significance was set at 5%.

Results

Patient characteristics

The HIV+LD subjects were comparable to HIV group with respect to age and body mass index. (age 47 (range 36 – 58) years and 53 (range 39 –64) years, p= 0.3; BMI 24.9 (17.6-32.2) and 23.3 (range 21.6-26.5), p= 0.45 in the HIV+LD and HIV-group, respectively).There also was no difference in amount of fat and fat free mass as measured by BIA. (fat mass 13.9 (range 7.2-20.8) kg and 16.4 (range 8.5-24.4) kg, p= 0.7; fat free mass 63.5 (range 47.2-84.5) kg and 54.2 (range 50.1 –61.5) kg, p= 0.06 in the HIV+LD and HIV-group, respectively). All LD patients had HIV-1 viral loads below 50 copies/ml at the time of assessment. The mean CD4 cell count was 510 cells /mm^3 (range 270- 980) in the LD group and 390 cells /mm^3 (range 10- 1130) in the control group (p = 0.63). Antiretroviral drug history, details of the regimens used, and body fat changes at enrollment for each of the eight LD subjects are shown in the table below.

Table 1 Antiretroviral treatment (ART) of the eight subjects from the LD group, all previous used ART and body fat changes.

	current ART regimen	months on current ART	previously used ART	body fat changes
1	AZT/3TC/RTV (100 mg bid) /IDV (800 mg bid)	2	AZT/ddI/ IDV	accumulation and atrophy
2	d4T/3TC/NFV (1250 mg bid)	28	none	accumulation and atrophy
3	d4T/3TC/ RTV (400 mg bid) /SQV (400 mg bid)	37	none	accumulation and atrophy
4	d4T/3TC/ IDV(800 mg tid)	34	none	atrophy only
5	d4T/3TC/ RTV (100 mg bid) / IDV (800 mg bid)	14	AZT/ddC/ IDV/NFV	accumulation and atrophy
6	d4T/3TC/ NFV (1250 mg bid)	17	RTV/SQV/IDV	accumulation and atrophy
7	d4T/ 3TC/ IDV	22	AZT	accumulation and atrophy
8	d4T/3TC/ RTV (100 mg bid) / IDV (800 mg bid)	14	none	accumulation and atrophy

If not mentioned explicitly standard dosage is being used. bid: twice-daily, tid: three times-daily, qd: once daily. ZDV: zidovudine 300 mg bid, 3TC: lamivudine 150 mg bid, d4T:stavudine 40 mg bid, ddI: didanosine 400 mg qd, ddC: zalcitabine 0,75 mg bid, RTV: ritonavir, IDV: indinavir, SQV: saquinavir, NFV: nelfinavir. atrophy: peripheral lipoatrophy, accumulation: central fat accumulation

Results in the postabsorptive state

Fasting plasma insulin and norepinephrine concentrations were significantly higher in the LD group, compared to the HIV group (p = 0.03 and p= 0.005, respectively). There was no difference in plasma epinephrine concentrations between the groups (Table 2). The postabsorptive rate of appearance of glycerol per kg body weight did not differ between the HIV and LD groups (Figure 1), nor was their a difference in the Ra glycerol expressed per kg fat mass between the two groups. There were no differences in systolic and diastolic blood pressure or pulse rate (data not shown). The median basal resting energy expenditure adjusted for lean body mass was 27% lower in the LD when compared with the HIV group (p = 0.002) (figure 2).

Table 2 Plasma hormone concentrations at baseline and after 60 minutes of epinephrine infusion

	LD		HIV	
	t=0	t=60	t=0	t=60
epinephrine (nmol/l)	0.09 (0.06-0.19)	1.54 (1.30-1.70)	0.16 (0.10-0.36)	1.32 (0.84-1.65)
norepinephrine (nmol/l)	1.61 (1.10-1.71) *	1.69 (1.43-2.38)	0.79 (0.67-0.96)	1.13 (0.95-1.39)
insulin (pmol/l)	105 (50-155) **	100 (60-200)	42 (28-49)	66 (35-76)

Data are median and interquartile ranges. * p-value = 0.005 compared to t=0 from control group, ** p-value= 0.03 compared to t=0 from control group.Nmol: nanomol , pmol: picomol

Results during epinephrine infusion

In both groups Ra glycerol per kg body weight increased. The response over time in the LD group was delayed when compared to the control group (p < 0.001) (figure 1). The prox mix model used showed no significant difference in overall response between the two arms, indicating that the areas under the curve were not different (p = 0.52). This same pattern was observed when we compared Ra glycerol expressed per kg fat mass between the two groups (p= 0.52). There was no difference in systolic and diastolic blood pressure over time between the two groups. The pulse rate increased slightly in both groups, but

there was no difference in the response between the groups over time. Plasma norepinephrine concentrations remained higher in the LD group during the whole experiment compared to the control group (p = 0.009)(Table 2, figure 3). The REE increased equally by ~ 10% in both groups (p = 0.59).

Figure 1 Total Ra glycerol per kg body weight and lipolytic response to epinephrine

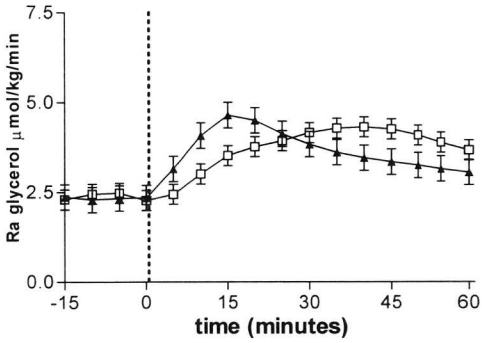


Figure 2 Mean resting energy expenditure during basal conditions and during epinephrine infusion

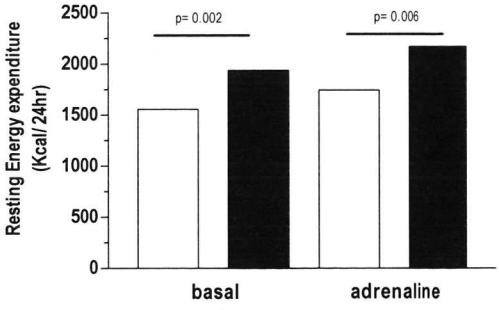
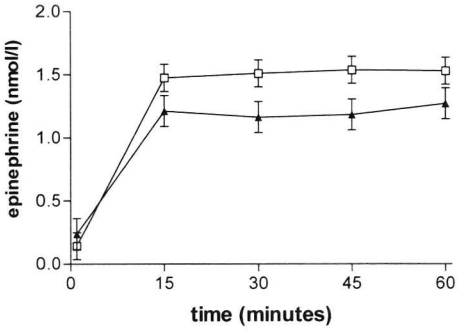
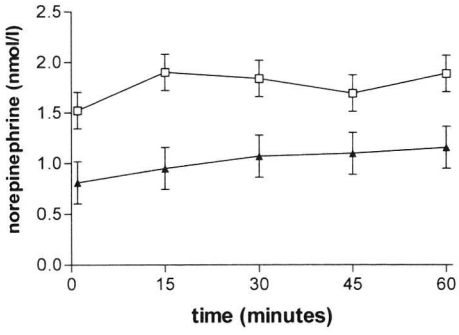


Figure 3 Epinephrine and norepinephrine concentrations during epinephrine infusion.





Open squares = LD group ; closed triangle = HIV group. µmol: micromol, kg: kilogram.Data are least square means ± standard errors. The p-value represents the outcome of a linear regression model adjusted for the amount of lean body mass in kg.

Discussion

HAART-related lipodystrophy is associated with subtle changes in endocrine regulation and in substrate and energy metabolism. In a previous study we found that the suppressive effect of insulin on plasma FFA levels was decreased. In the current study basal plasma glycerol turnover was not decreased in the postabsorptive state despite higher insulin concentrations, also suggesting insulin resistance. In addition plasma norepinephrine concentrations were increased. The lipolytic response to epinephrine was delayed although the qualitative response per se was not affected. Finally, the subjects with lipodystrophy had a lower REE compared to the HIV-control group.

We previously found that plasma FFA concentrations are increased in treatment-naive HIV-infected patients when compared to healthy volunteers [7] We chose to measure glycerol turnover in the current study to measure lipolysis more reliably. In the present study, basal lipolysis was not different in HAART treated subjects with lipodystrophy compared to untreated HIV-infected subjects. The increased rate of basal glycerol turnover found in both groups in our study, when compared to healthy controls in whom we have previously shown that basal lipolysis was significantly lower[7], is similar to that previously found in HIV-negative subjects with severe obesity (BMI 39 kg/m^2).[16] Just as in our HAART patients, in obesity the lipolytic response to epinephrine seems to be delayed when compared to that in lean subjects. [17] Moreover, in obese subjects a pancreatic clamp, which inhibits endogenous insulin production, increases glycerol turnover by approximately 50%. [18]. The similarity of these findings in obese subjects and our lipodystrophy patients suggest that HAART itself is not responsible for the delayed response in Ra glycerol. The higher concentrations of insulin found in the HIV+LD group during epinephrine infusion therefore may very well be directly or indirectly responsible for the observed delayed increase in lipolysis. One other important factor that might account for the delayed response is a

responsible for the observed delayed increase in lipolysis. One other important factor that might account for the delayed response is a difference in the perfusion of visceral adipose tissue between the groups. Adipose tissue blood flow plays an important role in the regulation of lipolysis.[19] There is a difference between the blood flow stimulating capacity of catecholamines in different fat compartments [20]. This difference is influenced by various tissue parameters, such as the size of adipocytes and differences in the organization and permeability of the connective web surrounding the adipocytes. A rise in plasma catecholamines results in an increase in lipolysis and a decrease in local blood flow[20]. Although never examined in lipodystrophy, one can hypothesize, that an increased size of omental adipocytes and a reduced fluid circulation in the visceral fat compartment may result in a decreased delivery of epinephrine to the adipocytes and a slower appearance of glycerol in the systemic circulation in subjects with lipodystrophy. The observation that overall lipolysis after 60 minutes of epinephrine infusion was not different in both groups, is compatible with such a mechanism both in our HAART patients and in obese patients in general.

The lipolytic response to epinephrine was delayed, which indicates impairment in the stimulation of hormone sensitive lipase (HSL) by epinephrine in HIV-1 infected patients with lipodystrophy. HSL is activated by binding of catecholamines to adrenergic receptors (AR) and inhibited by insulin[21,22]. A differential expression of AR on the cell surface of adipocytes results in different rates of lipolysis. Binding of catecholamines to $\beta_{1,2\text{ or }3}$- AR stimulates lipolysis while simultaneous activation of α_2–AR can partly impair this stimulating effect [23,24]. Increased α_2-AR activity in the visceral compartment in HAART-treated subjects with lipodystrophy could therefore account for the delayed lipolytic response. However this is unlikely because both in patients with obesity and patients with the metabolic syndrome the lipolytic response of omental adipocytes seems to be enhanced rather than decreased.[25,26].

One other interesting finding is the fact that plasma norepinephrine concentrations in the patients with lipodystrophy were almost twofold higher when compared to the controls, which has also been described by Renard ea [27], suggesting increased sympathetic nervous system activity in patients with lipodystrophy. The plasma concentration of norepinephrine reflects the resultant of production in the adrenal glands, (increased production seems an unlikely explanation for the increase in plasma concentration since plasma epinephrine concentrations did not differ at baseline) and the balance between release and re-uptake in sympathetic nerve endings. Another possible explanation could be a decreased clearance of norepinephrine due to competition with antiretroviral drugs, which to our knowledge has never been described. Therefore we believe that the most likely explanation for the increased plasma norepinephrine concentration in the subjects with lipodystrophy is increased sympathetic activity. Assuming this is correct, this could imply that the mechanism underlying the increased lipolysis in HIV-infection differs in different circumstances. In patients receiving no or inadequate treatment without lipodystrophy the mechanism is unknown, but unrelated to insulin or catecholamines. Tumor necrosis factor (TNF) is a good candidate, as it is a stimulator of lipolysis [28] and TNF production is increased in the natural course of HIV[29,30]. In our study lipolysis was similarly elevated in both groups indicating that, although the factor stimulating lipolysis in uncontrolled HIV infection had disappeared, it had been replaced by another mechanism, possibly a HAART associated increase in sympathetic activity.

To our surprise REE normalized in the HIV+LD group despite the increase in plasma norepinephrine concentrations. These data are in contrast with those previously published by Shevitz ea. In a large longitudinal study they found a strong positive correlation between the use of HAART and the REE, independent of HIV-1 RNA levels. However there is no documentation that these subjects were suffering

from lipodystrophy.[31] We measured fat free mass in both groups using BIA. This could lead to an overestimation of FFM since BIA mainly measures resistance and reactance in the extremities, which is likely to be increased more in the lipodystrophy group since these patients had severe lipoatrophy of their limbs. In the lipodystrophy group we did also measure FFM using whole body dual-energy X-ray absorptiometry (DEXA) as a tool to monitor any improvement in body appearance after the protease inhibitor withdrawal. FFM measured by DEXA indeed was 4.2 kg lower when compared to BIA ($p=0.009$) in this group with a 97% correlation between the two measurements. Therefore we calculated the REE adjusted for FFM using the DEXA data in the lipodystrophy group and the BIA data in the control group. The linear regression model used showed that REE adjusted for FFM was significantly lower in the lipodystrophy group when compared to the controls ($p=0.002$).
Taken together these data suggest that HIV-lipodystrophy counteracts the HAART-associated hypermetabolic state, which is already known to be elevated by HIV-infection. The absence of an increase in REE despite an increased sympathetic activity could be explained by selective sympathetic stimulation of the adipose tissue, as this organ contributes little to basal REE.

In summary, basal lipolysis in patients with lipodystrophy was not different when compared to patients with no (adequate) antiretroviral therapy in the presence of increased plasma insulin concentrations, indicating lipolysis to be resistant to the suppression by insulin. The lipolytic response to epinephrine infusion in patients with lipodystrophy was normal albeit delayed. Plasma norepinephrine concentrations were increased in patients with lipodystrophy, indicating increased sympathetic activity. The fasting REE was lower and remained lower during epinephrine infusion in the patients with lipodystrophy. This suggests that HAART-associated lipodystrophy as a result of concomitant sympathetic stimulation of adipose tissue has only minor effects on changes in lipolysis induced by HIV infection itself, but normalizes REE.

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