EVALUATION OF GLUCOSE UPTAKE BY SKELETAL MUSCLE TISSUE AND SUBCUTANEOUS FAT IN HIV-INFECTED PATIENTS WITH AND WITHOUT LIPODYSTROPHY USING FDG PET

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ABSTRACT

Objective: To evaluate differences in glucose uptake by skeletal muscle tissue and subcutaneous fat in HIV patients on HAART (**highly active antiretroviral therapy**)

presenting with and without lipodystrophy as well as in drug-naïve HIV patients using FDG PET.

Patients and methods: Thirty-nine consecutive patients suffering from HIV, respectively 7 drug-naïve patients, 21 non-lipodystrophic patients on HAART and 11 patients on HAART, suffering from lipodystrophy were prospectively included. All patients underwent a whole-body FDG PET examination. Standardized uptake values (SUV values) of muscle and subcutaneous fat were compared and related to demographic and biochemical variables.

Results: SUVmean values of subcutaneous fat were significantly higher in patients under HAART presenting with lipodystrophy when compared to untreated on treated, non-lipodystrophic, patients (p=0.000). SUVmean values of subcutaneous fat significantly correlated with treatment duration (r=0.56, p=0.000) and CD4 count (r= 0.51, p=0.001) and inversely correlated with viral load (r=-0.61, p= 0.000). Finally, SUV mean values of thigh muscles were not significantly different between the 3 different patient groups under study.

Conclusion: Quantitative FDG uptake by subcutaneous fat proved significantly higher in HIV patients under HAART presenting with lipodystrophy. HAART did not influence FDG uptake by human skeletal muscle tissue under basal conditions.

Key words: FDG PET-HAART-lipodystrophy

INTRODUCTION

Many patients infected with the human immunodeficiency virus (HIV) treated with highly active antiretroviral therapy (HAART) develop a syndrome, termed lipodystrophy, that is similar to the central/visceral obesity-insulin resistance syndrome which is characterized by a shift in body fat distribution to the central abdominal region, insulin resistance, hyperinsulinemia, dyslipidemia and in some, type 2 diabetes (1,2).

The underlying cause of the lipdoystrophy syndrome in HIV-patients on HAART is currently unclear. One theory is that HAART interferes with the body's processing of fat. Lipodystrophy in HIV-infected patients is characterized by subcutaneous fat atrophy and visceral fat accumulation (3,4). Some degree of apoptosis, in the absence of significant inflammatory phenomena, occurs in subcutaneous adipocytes from patients on HAART-associated lipodystrophy, providing evidence for a possible mechanism of subcutaneous adipocyte loss in these patients (5). Apoptosis is an energy dependent phenomenon that is accompanied by increased FDG utilization as evidenced by tumour xenograft models (6,7).

Another theory is that insulin resistance plays a causative role in lipodystrophy (8,9,10). Glucose transport is a critical step for insulin-stimulated glucose utilization in skeletal muscle. The process of insulin-stimulated glucose transport requires the movement of intracellular G glucose-transporter 4 (GLUT4) –rich vesicles to the cell surface, accompanied by translocation of additional vesicular cargo proteins. Several reports in animals indicate that HAART can decrease the intrinsic activity of the glucose transporter GLUT-4, resulting in insulin resistance and a decrease of glucose uptake by skeletal muscle tissue (11). Insulin resistance by skeletal muscle cells will result in hyperinsulinemia and hyperinsulinemia was noted to be associated with obesity already shortly after the development of the assay for

insulin. Since than, this association has been confirmed with the hyperinsulinemic-euglycemic clamp and with the frequently sampled intravenous glucose tolerance test.

18-Fluorodeoxyglucose (FDG) positron emission tomography (PET) is a well validated technique to image and measure uptake of glucose in normal tissues, cancer and inflammatory lesions. In this study, FDG PET was used to assess differences in glucose uptake by skeletal muscle tissue and subcutaneous fat in HIV patients on HAART presenting with and without lipodystrophy as well as in .drug-naïve HIV patients. **If FDG PET allows quantitative detection of lipodystrophy in HIV patients, it may be used for quantitative non-invasive monitoring of therapy response to treatment options aimed at reducing insulin-resistance in these patients.**

METHODS

Patients

Thirty-nine consecutive patients suffering from HIV-1, 7 of which were drug-naïve, were prospectively included in the study. Of the remaining 32 patients under HAART treatment, eleven patients presented with lipodystrophy. The diagnosis of HIV-related lipodystrophy was based on the following criteria: clinical evidence by physician's and patients' reports of fat wasting from the face, buttocks, limbs, and upper trunk with accumulation of fat in the abdomen or over the dorsocervical spine and fasting hyperlipidaemia (cholesterol $\geq$ 5.5 mmol/l or triglyceride $\geq$ 2.0 mmol/l), fasting C-peptide greater than 2.5 mmol/l or impaired fasting glucose or diabetes mellitus (fasting glucose > 6.1 mmol/l) in the absence of an AIDS-

defining event. None of the patients had received anabolic steroids, glucocorticoids or immune modulators within 3 months of assessment. Additional variables recorded included demographic features, antiretroviral therapies, CD4 cell count and plasma HIV-1 RNA levels at the time of imaging. All patients gave their written informed consent and the study was approved by the institutional review board of the University Hospital of Pretoria, South-Africa.

All patients underwent a whole-body FDG PET examination. Standardized uptake values of muscle and subcutaneous fat were obtained by FDG PET imaging and compared between the three groups.

FDG PET/CT scanning

Whole body 18-FDG PET scans were acquired on a dedicated PET-CT scanner (Biograph, Siemens) from the skull base to the pelvis. Patients were required to be fasting for a minimum of 4 hours prior to FDG injection, inducing a low insulin state. Blood glycaemia was monitored with a portable capillary glucometer. Patients received a dose of FDG based on their body weight using the following formula ((body weight/10)+1))*37 MBq. PET/CT imaging was performed 45 min after injection of FDG. Acquisition duration per bed position was 3 min; nine bed positions were acquired.

Images were acquired in a 3-dimensional mode and reconstructed with and without attenuation correction (CT-based) using OSEM (ordered subset expectation maximization) yielding axial, sagittal and coronal slices. FDG SUV mean values of thigh muscle and subcutaneous abdominal fat (belly, supra-umbilical) were derived from the reconstructed PET images using square regions of interest positioned based on the CT-scan findings. Regions of

interest positioning was performed by two nuclear medicine specialist blinded to the clinical and biochemical data. Averaged data were used for further analysis.

Statistical analysis

Statistical analysis was performed using SPSS version 15.0.

Correlation analysis was performed using the non-parametric Spearman-rank test. Normality was assessed using the Kolmogorov-Smirnov test.

Differences in the variables studied between the three groups under study were assessed by means of the Kruskal-Wallis test and additional Mann-Whitney testing considering p-values inferior to 0.01 as statistically significant in order to correct for multiple comparisons.

RESULTS

Patient data are shown in table 1.

Median age of the patients in the therapy-naïve group (3men/4 women) was 34.9 years (range: 24.7-64.2 years). Median age of the patients under HAART without lipodystrophy (7 men/14 women) was 38.4 years (range: 16.7-62.1 years). Three different treatment regimens were used, regimen 1 (R1) consisted of stavudine, lamivudine and efavirenz, regimen 2 (R2) consisted of stavudine, lamivudine and nevarapine and regimen 3 (R3) consisted of stavudine, didanosine and efavirenz. Of the patients under HAART without lipodystrophy, seventeen patients were on R1, 2 on R2 and 2 on R3. Median age of the patients under HAART with lipodystrophy (8 men/3 women) was 37.3 years (range: 23.0-53.2 years). Eight patients were on R1, one patient on R2 and two patients on R3.

Median duration of HAART was 5 months (range 0.5-24 months) in treated patients without lipodystrophy and 12 months (range 6-24 months) in treated patients with lipodystrophy.

Median values for viral load (copy number/mL) and CD4 cell count (/mL) were respectively 457000/mL(range: 223458-532175 /mL) and 59/mL(range: 53-117/mL) in therapy naïve patients, 972/mL(range: 50-321543/mL) and 256/mL(range: 50-524/mL) in treated patients without lipodystrophy and 188/mL(range: 50-154234/mL) and 256/mL(range: 53-542/mL) in treated patients with lipodystrophy.

SUV mean values of subcutaneous fat were not significantly different between therapy-naïve patients and patients under HAART that did not present with lipodystrophy. In contrast, SUVmean values of subcutaneous fat were significantly higher in patients under HAART presenting with lipodystrophy when compared to both other groups (p=0.0001)(also see Figure 1). Mean values and standard deviations obtained for SUVmean of subcutaneous fat for the three different groups were respectively: 0.34(sd:0.14) for the therapy naïve group, 0.46(sd:0.24) in patients under HAART not presenting with lipodystrophy and 0.9(sd: 0.14) in the patients under HAART that presented with lipodystrophy. **Thus it appears that FDG uptake in subcutaneous fat is increased because of lipodystrophy, not because of HAART.** SUVmean values of subcutaneous fat were correlated with treatment duration (r=0.56, p=0.0001) and CD4 count (r= 0.51, p=0.001) and inversely correlated with viral load (r=-0.61, p= 0.0001).

SUV mean values of thigh muscles were not significantly different between the different groups under study. Respective values obtained were; 0.59(sd:0.15) for the therapy naïve group, 0.62(sd:0.17) in patients under HAART not presenting with lipodystrophy and 0.63(sd: 0.18) in the patients under HAART that presented with lipodystrophy.

DISCUSSION

This study aimed to assess potential differences in quantitative FDG uptake by skeletal muscle tissue and subcutaneous fat in HIV patients on HAART presenting with and without lipodystrophy as well as in .drug-naïve HIV patients.

Based on data derived from light microscopic and ultra-structural observations in a limited number of HIV-patients under HAART, some authors have suggested that lipo-dystrophy results from **replacement of** white fat **by brown fat** , which is highly FDG (12,13,14,15). However, hypermetabolic brown fat requires a high mitochondrial concentration and both protease inhibitors and nucleoside reverse transcriptase inhibitors, in particular stavudine, were shown to induce lipo-atrophy by causing mitochondrial dysfunction, mitochondrial depletion and by deleting mitochondrial DNA in adipocytes (16). In a series of fourteen HIV-infected patients with HIV-1 protease inhibitor-associated lipodystrophy by Domingo et al, biopsy of subcutaneous fat was performed in the antero-lateral aspect of the right leg of all patients under study (5). One of the eleven assessable biopsy samples was negative for the presence of apoptosis, six showed focally positive apoptotic cells, and the remaining four biopsies demonstrated moderate positivity. Apoptosis is an energy dependent phenomenon that is accompanied by increased FDG utilization, accordingly, the increase in SUV mean of subcutaneous fat of patients suffering from HIV-related lipodystrophy under HAART therapy may theoretically reflect ongoing apoptosis. Our findings demonstrating a positive correlation between CD4 cell count and an inverse correlation with viral load are in line with the findings by Domingo et al.

As shown by Cherry et al. and McComsey et al., apoptosis of fat cells is a process that my be reversed or reduced through HAART dose reduction or modification (17,18). In line with these findings, using visual analysis of FDG PET images a significant reduction of FDG

uptake was observed in three patients in whom stavudine was replaced by the less mitochondrial toxic zidovudine, abacavir or tenovir by Bleeker-Rovers et al (19). Their and our findings taken together suggest that FDG PET may allow for quantitative non-invasive monitoring of lipo-atrophy of subcutaneous fat during the course of HIV treatment using currently available treatment options but especially novel treatment options aimed at reducing **lipodystrophy and insulin-resistance**.

Behrens et al previously aimed to identify possible defects in skeletal muscle glucose uptake and metabolism in HIV patients receiving HAART using **a combination of the euglycemic-hyperinsulinemic clamp technique, indirect calorimetry and dynamic FDG PET imaging of the thighs** (20). Whole-body glucose disposal was significantly reduced in patients presenting with lipodystrophy when compared with untreated patients. Reduced skeletal glucose uptake was caused by significantly impaired glucose transport and phosphorylation; skeletal muscle glucose uptake was reduced by 66% in treated patients when compared to therapy-naive patients. Contrary to these findings obtained under insulinic stress, available preclinical data suggest that under basal conditions, HAART increases rather than decreases glucose uptake of skeletal muscle cells (21). More specifically, both saquinovir, indinavir, ritonavir and amprenavir at a concentration of 10 μM were shown to nearly double the uptake of 2-deoxyglucose of L6 myoblasts under basal conditions. Our findings, however, do not corroborate these preclinical findings; identical levels of FDG uptake were found in untreated and treated patients, either presenting with our without lipodystrophy.

In conclusion, quantitative FDG uptake by subcutaneous fat proved significantly higher in HIV patients under HAART presenting with lipodystrophy when compared to therapy naïve HIV patients or patients under HAART that did not suffer from lipodystrophy. Contrary to

available preclinical data, HAART did not influence FDG uptake by human skeletal muscle tissue under basal sedentary conditions. **The potential of FDG PET to monitor response to treatment options aimed at reducing insulin-resistance in lipodystrophic HIV-patients under HAART warrants further investigation.**

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Table 1.

	Therapy Naïve	HAART	HAART with lipodystrophy
Age (me/ra)	34.9yrs(27.4-64.2 yrs)	38.4yrs(16.7-62.1 yrs)	37.3yrs(23.0-53.2 yrs)
Gender(M/W)	3/4	7/14	8/3
TD(me/ra)	-	5M(0.5-24M)	12M(6-24M)
TR(R1/R2/R3)	0/0/0	17/2/2	8/1/2
VL(me/ra)	457000(223458-532176)	972(50-321543)	188(50-154234)
CD4(me/ra)	59(53-117)	256(50-524)	256(53-542)
SUV sc.Fat m/sd	0.34(0.14)	0.46(0.24)	0.9(0.15)
SUV Muscle m/sd	0.59(0.15)	0.62(0.17)	0.63(0.18)

me= median, ra= range, M= men, W= women, TD= treatment duration, TR= treatment regimen, R1= stavudine + lamivudine+ efavirenz, R2= stavudine, lamivudine and nevarapine and R3=stavudine, didanosine and efavirenz,VL= viral load, CD4= number of CD4+ cells, SUV= standardized uptake value, sc.= subcutaneous, m= mean, sd= standard deviation, HAART= highly active antiretroviral therapy.
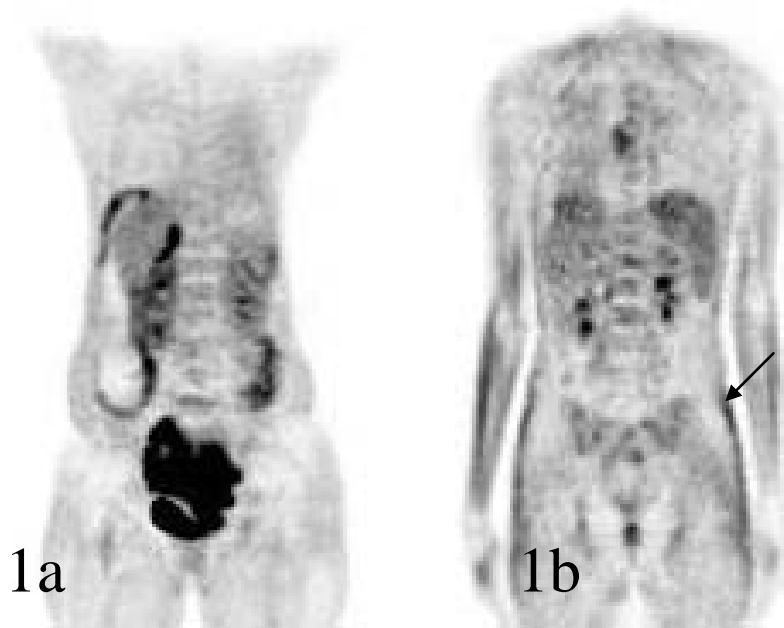
Figure 1.

Figure 1a shows a coronal image of a patient under HAART showing normal FDG uptake in subcutaneous fat. Figure 1b shows a coronal image of a patient under HAART with lipodystrophy with increased FDG uptake in subcutaneous fat, as indicated by a small arrow.