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Monika Bociąga-Jasik[1, A, B, C, D, F], Anna Polus[2, G], Joanna Góralska[2, C, G], Urszula Raźny[2, G], Dominika Siedlecka[2, G], Barbara Zapała[2, G], Robert Chrzan[3, G], Aleksander Garlicki[1, E], Tomasz Mach[1, E], Aldona Dembińska-Kieć[2, A, E, F]

# Impact of Antiretroviral Therapy on Selected Metabolic Disorders – Pilot Study

[1] Chair of Gastroenterology, Hepatology and Infectious Diseases, Jagiellonian University, Collegium Medicum, Kraków, Poland
[2] Department of Clinical Biochemistry, Jagiellonian University, Collegium Medicum, Kraków, Poland
[3] Department of Radiology, Jagiellonian University, Collegium Medicum, Kraków, Poland

**A** – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

## Abstract

**Background.** Taking into consideration the aging of HIV infected individuals, changes in the metabolism aggravated by the antiretroviral therapy significantly impact their health. Mechanisms responsible for lipodystrophy, dyslipidemia and insulin resistance (IR) occurrence have not been completely understood. Only recently, the free fatty acids (FFAs) metabolic turnover has become considered to be the independent risk factor for cardiovascular complications.
**Material and Methods.** We designed the follow-up study in which patients were recruited before the introduction of ARV therapy and then observed up to 1 year. The impact of ARV therapy on the development of metabolic complications, inflammation markers and changes in adipokines secretion was investigated. The fasting and postprandial responses of FFAs, triglycerides (TG), glucose, insulin and glucose-dependent insulinotropic peptide (GIP) were measured. Changes in body composition were followed by impedance and a CT scan of adipose tissue volume of the abdomen and thighs.
**Results.** Significant impact of ARV therapy on metabolic disturbances was reported. Not only fasting, but also postprandial levels of FFAs and TG were found to increase during the follow up.
**Conclusions.** The increased concentration of FFAs is suggested to be the triggering event in the development of hypertriglyceridemia and insulin resistance during ARV therapy. Changes in postprandial FFAs and TG during the follow up indicate the increasing risk of cardiovascular diseases. We conclude that modern ARV therapy during the period of 12 months does not induce changes in the fat distribution, although increased limb fat correlated with higher plasma leptin level, which may be the marker of increased risk of metabolic driven cardiovascular complications (**Adv Clin Exp Med 2014, 23, 4, 539–549**).

**Key words:** adipokines, FFAs, GIP, HIV, IL-6, postprandial test, TNFα.

Nowadays, HIV infection is considered a chronic condition, and therapy with ARV drugs significantly decreased the number of HIV related morbidity and mortality [1]. As the population of HIV infected patients is aging, we face new problems connected with increasing risk of metabolic and cardiovascular diseases, which can have a significant impact on the quality of patients' lives and can also decrease survival.

The pathogenesis of the above complications in HIV infected population is complex and not completely explored [2]. There is evidence that therapy with ARV drugs, especially nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs), is associated with side effects such as lipodystrophy, hyperlipidemia and insulin resistance [3]. It is suggested that HIV infection by

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itself can induce changes which are promote the development of metabolic disturbances [2, 4]. It has been documented that interrupting ARV therapy, and in consequence uncontrolled HIV replication, may further increase the risk of all-causes mortality by raising IL-6 and D-dimers level [5]. Also, the SMART trial found a raised risk of cardiovascular diseases in patients undergoing intermittent therapy, although the reasons of this remain unclear [6].

As variable changes in lipid profile were reported in ARV treated patients, little is known about the effect of HIV infection and ARV therapy on postprandial lipemia, which is considered to be an emerging cardiovascular diseases risk factor [7]. Insulin resistance is an element of metabolic disturbances which occur in HIV infected people [8]. The mechanism of insulin resistance is in part due to the disturbed lipid/FFAs metabolism and inflammation, which can promote lipotoxicity [8]. The postprandial, protective for beta-cells, incretin response to oral nutrients have also been poorly followed in the development of impaired glucose tolerance in the ARV treated patients. To investigate the pathogenesis of the above complications, we designed the follow up study in which HIV patients were recruited before the introduction of ARV therapy and then followed up to 1 year. The aim of the presented study was to assess the impact of ARV therapy on the development of metabolic complications, inflammation markers (TNFα, IL-6,), as well as changes in the endocrine function of the adipose tissue connected with secretions of selected adipokines (adiponectin, leptin and fatty acid binding protein 4 (FABP4)). The postprandial responses of free fatty acids (FFAs), triglycerides (TG), glucose, insulin, as well as glucose-dependent insulinotropic peptide (GIP) were measured.

# Material and Methods

## Characteristics of Patients

The study was approved by the Ethics Committee of the Jagiellonian University Collegium Medicum (No. KBET/52/B/2008). Written informed consent was obtained from all study participants.

HIV infected, ARV-naive patients were recruited from Outpatient Clinic of the University Hospital in Krakow. Patients with active opportunistic infections, neoplastic diseases, HCV and HBV coinfection, metabolic complications, body mass index (BMI) ≥ 25, as well as those smoking cigarettes and taking any other medications were excluded from the study. All patients at the moment of the enrollment into the study required the introduction of ARV drugs, and were qualified to the therapy according the HIV guidelines, on the basis of the provider doctor decision (Table 1). Only patients who, based on the provider decision, were offered therapy with regimen containing two nucleoside reverse transcriptase inhibitors (NRTIs) combined with one protease inhibitor (PI) regimen were included into the investigation. Overall 13 patients were recruited into the study, 11 (85%) men and 2 women (15%), aged 30.5 years (min. 23, max. 42). The following combinations of NRTIs were used in investigated patients: tenofovir/emtricitabine – 7 pts, tenofovir/ /lamivudine – 3 pts, abacavir/lamivudine – 3 pts. All PIs used in the study were boosted by ritonavir (r): 6 patients were treated with atazanvir/r, 5 with darunavir/r and 2 with lopinavir/r. Detailed characteristics of patients are presented in Table 1. The study was conducted for 12 months, and the blood was collected after 6 months, as well as after 12 months of above therapy. To monitor the therapy CD4 cell count and viral load were measured according to HIV guidelines. In all investigated patients, accordant immunological and virological response to the therapy was achieved (Table 1). None of the patients interrupted or changed their therapy during the observation period, but two of them refused to participate in the study after 6 months.

## Body Composition Assessment

Assessment of patients body composition, including: Body Fat Content (Body Fat % (B. FAT%), body fat mass (B.FAT kg)), body mass index (BMI), body impedance, resting metabolic rate (RMR), lean mass weight (Lean Kg), lean mass % (Lean %) was determined by Maltron BF-907 Analyzer. The anthropometric measurements, including: waist to hip ratio were performed. During the follow-up, patients were asked to fill in a self-assessment questionnaire regarding changes in their body composition.

To assess more accurate changes in the fat distribution connected with ARV therapy adipose tissue volume of abdomen and thighs was measured by CT examination and abdomen/thighs fat volume ratio was calculated. A CT scan was done at the moment of the enrollment into the study as well as 6 and 12 months later.

CT examinations were performed using a helical 16-row scanner Siemens Somatom Sensation 16.

After obtaining a lateral topogram of the abdomen, scanning of a single slice 10 mm thick at the level of the lower edge of L4 vertebral body was performed (voltage 120 kV, current 170 mA, reconstruction kernel B31s) – Fig. 1.

**Table 1.** Patients characteristic

| | **HIV infected- before ARV therapy (n-13)** | **HIV infected – 6 months after introduction of ARV therapy (n-13)** | **HIV- infected 12 mo after introduction of ARV therapy (n-11)** | **Friedman's Test** |
|---|---|---|---|---|
| CD4 cells/μL | 301 (± 78.2, min. 148 – max. 383) | 477.20 (± 162.22, min. 199–max. 696) | 565 (± 163.40, min. 249–max. 802) | Chi-square – 20.462 $p < 0.001$ |
| Viral load copii/mL | 278 351 (min. 5 700–max. 1 3100) | 206.6 (± 376.9, min. 34–max. 1440) | 17.77 (± 20.5, min. 0–max. 48) | Chi-square – 8.000 $p < 0.05$ |
| Body Mass Index (BMI) | 24 | 24.15 | 24.80 | Chi-square – 0.194 ns. |
| Waist to Hip ratio (WHR) | 0.89 (± 0.076, min. 0.75–max. 0.99) | 0.92 (± 0.08, min. 0.81–max. 1.08) | 0.92 (± 0.63, min. 0.80–max. 1.08) | Chi-square – 3.073 ns. |
| Body Fat (BFAT kg) | 16.58 (± 5.96, min. 8.9–max. 26.2) | 16.2 (± 6.21, min. 9–max. 29.9) | 16.73 (± 4.92, min. 10.6–max. 24.8) | Chi-square – 0.250 ns. |
| Impedance | 519.384 (± 64.9, min. 426–max. 622) | 501.5 (± 64.2, min. 373–max. 583) | 392.2 (± 183, min.–max. 573) | Chi-square – 0.356 ns. |
| Resting Metabolic Rate (RMR) | 1751.5 (± 171.5, min. 1466.0–max. 2089.0) | 1769, 6 (± 171. 4, min. 1475–max. 2054) | 1503.7 (± 691.6 min. 1530–max. 2127) | Chi-square – 1.077 ns. |
| CT adipose tissue volume at abdomen level [$mm^3$] | 230553.77 (± 87517.67, min. 98925–max. 362616) | 254847.92 (± 76139.82, min. 146532–max. 405397) | 247620.73 (± 115458, min. 128384–max. 376205) | Chi-square –1.077 ns. |
| CT adipose tissue volume at thigh level [mm3] | 111490.31 (± 63629.25, min. 58498–max. 266838) | 125494.08 (± 64191.25, min. 75703–max. 301495) | 124495.27 (± 91590.77, min. 71354–max. 288353) | Chi-square – 5.538 $p = 0.063$ |
| CT ratio of adipose tissue volume at abdomen/thigh [$mm^3$] | 2.27 (± 0.79, min. 1.28–max. 3.54) | 2.24 (± 0.80, min. 1.34–max. 3.58) | 2.24 (± 1.55, min. 1.30–max. 5.03) | Chi-square – 1.167 ns. |

ns. – non significant.

After obtaining an anterio-posterior topogram of thighs, a single slice 10 mm thick shaft center at the level of the right femur was scanned (voltage 120 kV, current 170 mA, reconstruction kernel B31s) – Fig. 2. Two axial scans were imported into 3D Slicer v. 4.1 (http://www.slicer.org) program[1] in DICOM format. Independently for both the above-mentioned images, the pixels representing

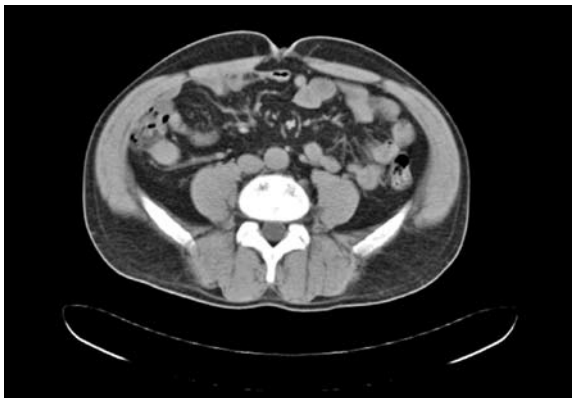

**Fig. 1.** CT – slice at the level of the lower edge of L4 vertebral body

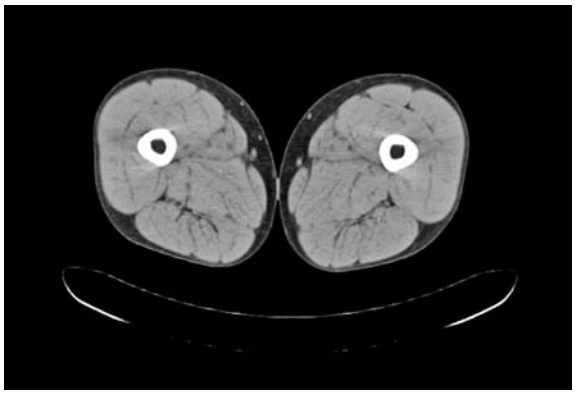

**Fig. 2.** CT – slice at the level of the right femur shaft center

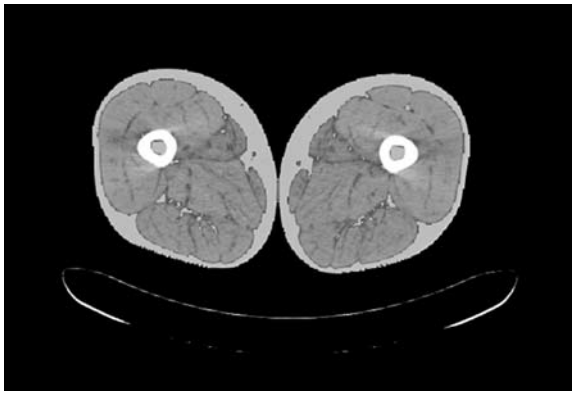

**Fig. 3.** CT – slice at the level of the lower edge of L4 vertebral body with adipose tissue segmentation

the table for the patient were manually removed in the program from the measurement region. Next, an automatic segmentation of body regions with adipose tissue attenuation was performed, assuming the range of values from –150 to –30 HU – Fig. 3. Finally, the program calculated volumes of the above-mentioned regions. The values obtained were documented as absolute numbers: adipose tissue volume in a slice at the level of abdomen [$mm^3$], adipose tissue volume in a slice at the level of thighs [$mm^3$] and ratio of these values [9].

## Laboratory Analysis

CD4 cells count was analyzed by a flow cytometer and HIV-1 load was determined by the HIV Cobas Ampliprep CAP-CTM-HIV using the Cobas Ampilcor system (Roche Diagnostic).

Changes in lipid profile (fasting lipidogram (total cholesterol (TC), low-density lipoprotein (LDL), triglycerides (TG), high-density lipoprotein (HDL) were tested before ARV therapy was introduced, as well as 6 and 12 months thereafter. In addition, an oral lipid tolerance test (OLTT) was performed according to Tanaka et al. [10]. Baseline blood samples were collected after 10 hours overnight fasting to assess fasting: triglycerides (TG), free fatty acids (FFAs), glucose, insulin, incretin (GIP) levels, and then patients ingested a high-fat breakfast. Venous blood samples were collected after 2 and 4 h, for retesting the above-mentioned parameters.

Fasting plasma glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL) were estimated by usual enzymatic procedures. FFAs were measured in non-frozen plasma by enzymatic colorimetric method (Roche Applied Science). Fasting insulin level was measured by immunoradiometric method (Diasource Immunoassys) and read-out using the gamma counter LKB Instruments. Method sensitivity was 1 μIU/mL, precision within the assay 2.1% and inter assay precision 6.5%.

Insulin resistance index (HOMA-IR) was calculated on the basis of the following formula: HOMA-IR = fasting insulin μU/mL) x fasting plasma glucose (mmol/l)/22.5. HOMA-IR above > 2.5 was considered to define insulin resistance.

Fasting GIP concentration was measured using ELISA kit (Millipore Corporation, accuracy, 86.7 ± 3%, precision: inter assay 1.8–6.1% and inter assay 3.0–8.8%).

To assess TNFα, IL-6, adiponectin, leptin and FABP4 concentrations, 0.5 mL of serum was immediately frozen and stored up to 12 months at –80°C. Serum levels of TNFα and IL-6 were measured by high sensitivity ELISA tests (R&D System) according to manufacturer protocol with sensitivity 0.12 pg/mL, 0.04 pg/mL respectively, and precision within the assay was expressed as coefficient of variation (CV) around 6% and 7%. Inter assay precision was around 10%, and 8%.

To assess serum leptin and adiponectin (human total adiponectin/Acrp30) immunoenzymatic assays (R&D System) with sensitivity 7.8 pg/mL and 0.25 ng/mL respectively were used. CV was 3% and 4%, and inter assay precision around 4% and 6%.

FABP4 concentration was evaluated by immunoenzymatic method (BioVendor), with a sensitivity of 0.05 ng/mL, CV around 2.5%, and inter assay precision around 4%.

## Statistical Analysis

The results are shown as mean ± standard deviation (SD). The comparisons between investigated groups were performed using the Anova Friedman's test. To carry out a pairwise comparison, a *post hoc* analysis for the Friedman's test was done for two related samples. To measure the oral lipid tolerance (OLTT) test, the area under the curves (AUCs) were calculated by the trapezoidal rule. Correlations were assessed using the Pearson test and r coefficients were shown. p-value < 0.05 was considered as statistically significant. Analysis of the results was carried out using the SPSS version 21.

## Results

### Metabolic Changes During the 12 Months Follow-up Study

During the 12 months of the follow-up, we did not observe clinically explicit lipodystrophy in investigated HIV infected patients, and there were no

statistically significant changes in waist to hip ratio (WHR), as well as the ratio of adipose tissue volume in a slice at the level of the abdomen [mm$^3$] to adipose tissue volume in a slice at the level of thighs [mm3] (Table 1). The tendency (Chi-square –5.538, p = 0.063) of adipose tissue volume in a slice of the thigh to increase was observed during this period (Table 1). There was an increase of adipose tissue volume at the level of the thigh after 6 months of ART as compared with the baseline ($p < 0.05$). Patients (n = 3), who in the self-assessment questionnaire reported changes in body composition, had an increase of ratio of adipose tissue volume at abdomen to thigh level at the 12$^{th}$ month.

The CT adipose tissue volume at abdomen level [mm$^3$] at the baseline, 6 and 12 months positively correlated with leptin level ($r = 0.7368$, $p < 0.01$ vs. $r = 0.6711$, $p < 0.05$ vs. $r = 0.7142$, $p < 0.05$). There was also a positive correlation with FABP4 at month 6$^{th}$ and 12$^{th}$ of the follow-up ($r = 0.5534$, $p < 0.05$ vs. $r = 0.7024$, $p < 0.05$).

The adipose tissue volume at the abdomen level [mm$^3$] measured by CT at month 12$^{th}$ positively correlated with the total cholesterol (TC) ($r = 0.7238$, $p < 0.05$), insulin level ($r = 0.6568$, $p < 0.05$) and HOMA-IR ($r = 0.7219$, $p < 0.05$). Also, adipose tissue volume at thigh level [mm$^3$] measured by CT during the whole period of the follow-up positively correlated with leptin level ($r = 0.9423$, $p < 0.001$vs. $r = 0.7362$, $p < 0.01$ vs. $r = 0.8899$, $p < 0.001$).

CT ratio of adipose tissue volume at abdomen/thigh [mm3] positively correlated with FFAs level ($r = 0.6826$, $p < 0.05$) at baseline and with insulin level ($r = 0.8781$, $p < 0.001$), HOMA-IR ($r = 0.9043$, $p < 0.001$) and FABP4 concentration ($r = 0.7515$, $p < 0.001$) after the 12 months of observation.

There was a negative correlation of the changes of adipose tissue volume at thigh level [mm$^3$] (12 months minus baseline) with the LDL ($r = –0.7471$, $p < 0.01$) (Fig. 4.) and a positive correlation with TG level ($r = 0.6791$, $p < 0.05$). We have not observed changes in BMI, body fat composition (BFAT kg) and RMR (Table 1.), though body fat mass measured by impedance method was lower after 12 months as compared with the baseline and after 6 months of ART ($p < 0.05$).

There were no changes in the lipid profile of investigated patients before the introduction of ARV therapy, although 6 months later 4 (31%) of them developed dyslipidemia; 1 (8%) had mixed dyslipidemia and 3 (23%) of the patients had an elevated triglycerides level (> 2.2 mmol/l).

The similar pattern of metabolic changes was observed 12 months after the introduction of therapy. There were statistically significant changes in TC and TG concentrations in the investigated patients during the follow-up, as compared with baseline values. Detailed characteristics of changes in lipid profile during the study were presented in Table 2.

The performed analysis reveals a significantly higher TG level after 12 months of therapy as compared with TG at the moment of the recruitment into the study ($p < 0.05$).

Before the ARV therapy was introduced, 3 patients (23%) had insulin resistance (IR), 6 months later 5 patients (38.5%) had HOMA-IR > 2.5 as compared with 3 (27%) patients at month 12$^{th.}$

There were no differences in IR, as well as in glucose and insulin concentration during the 12 months of the follow-up (Table 2).

Fasting and postprandial glucose, insulin, GIP, FFAs, TG concentrations at baseline and 6 and 12 months after the introduction of ART are presented in Table 3.

Statistically significant changes in fasting FFAs concentrations were observed during the follow-up ($p < 0.05$) (Table 4). The performed analysis confirmed statistically higher fasting FFAs 12 months after the introduction of ARV therapy, as compared with fasting FFAs at baseline ($p < 0.05$). Not

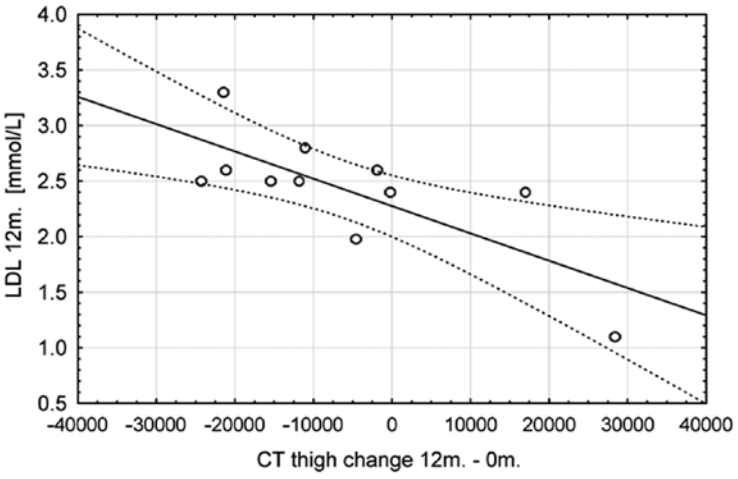


**Fig. 4.** Correlation of adipose tissue volume at thigh level with LDL-cholesterol after 12 months of therapy. Negative correlation of adipose tissue volume at thigh level [mm$^3$] (12 months minus baseline) with LDL-cholesterol level after 12 months of ARV therapy ($r = –0.7471$, $p < 0.01$)

**Table 2.** Metabolic changes during the 12 months of ARV therapy

| | Before introduction of ARV therapy – 0 | 6 months after introduction – I | 12 months after introduction – II | Friedman's test |
|---|---|---|---|---|
| Total Cholesterol (TC) mmol/l | 4.04 (± 0.7, min. 3.21–max. 5.1) | 4.78 (± 0.54, min. 3.84–max. 5.7) | 4.84 (± 0.52, min. 4.2–max. 5.6) | Chi-square – 6.837 $p < 0.05$ |
| Low-density lipoprotein (LDL) mmol/l | 2.82 (± 0.6, min. 0.6–max. 3.1) | 2.56 (± 0.44, min. 1.92–max. 3.4) | 2.43 (± 0.82, min. 1.1–max. 3.3) | Chi-square – 4.000 ns. |
| High-density lipoprotein (HDL) mmol/l | 1.14 (± 0.2, min. 0.8–max. 1.4) | 1.33 (± 0.31, min. 0.96–max. 2.0) | 1.36 (± 0.32, min. 1.0–max. 1.9) | Chi-square – 3.674 ns. |
| Triglycerides (TG) mmol/l | 1.21 (± 0.40, min. 0.19–max. 1.4) | 1.85 (± 0.68, min. 0.8–max. 3.6) | 2.34 (± 0.90, min.1.2–max. 3.6) | Chi-square – 7.091 $p < 0.01$ |
| Free fatty acids (FFAs) mmol/l | 0.71 (± 0.41, min. 0.19–max. 5.6) | 0.65 (± 0.31, min. 0.23–max. 1.38) | 1.0 (± 0.14, min. 0.53–max. 2.19) | Chi-square – 6.000 $p < 0.05$ |
| Fasting glucose mmol/mL | 4.83 (± 0.37, min. 4.2–max. 5.6) | 4.98(± 0.89, min. 4.0–max.6.2) | 4.65(± 0.47, min. 4.3–max. 5.4) | Chi-square – 0.571 ns. |
| Insulin μU/mL | 9.44 (± 3.14, min. 6.1–max. 17.9) | 13.22 (± 9.16, min. 6.6–max. 39.2) | 10.24 (± 6.27, min. 5.6–max. 20.7) | Chi-square – 1.163 ns. |
| HOMA-IR | 2.03 (± 0.73, min. 1.27–max. 3.90) | 3.11 (± 2.88, min. 1.35–max. 12.2) | 2.10 (± 0.88, min. 1.24–max. 4.4) | Chi-square – 0.545 ns. |

Data are presented as mean (± SD, minimum–maximum).

**Table 3.** Changes in cytokines and adipokines concentrations during the 12 months of ARV therapy

| | Before introduction of ARV therapy – 0 | 6 months after introduction – I | 12 months after introduction – II | Friedman's test |
|---|---|---|---|---|
| TNFα pg/mL | 2.89 (± 1.88, min. 1.05–max.12.44) | 1.46 (± 0.62, min. 90–max. 3.37) | 1.38 (± 0.73, 0.93–3.54) | Chi-square – 11.455 $p < 0.01$ |
| IL-6 pg/mL | 1.85 (± 1.83, min. 0.52–max. 7.55) | 1.09 (± 0.44, min. 0.60–max. 2.11) | 1.62 (± 1.70, min. 0.47–max. 5.76) | Chi-square – 3.455 $p = 0.1$ |
| Adiponectin ug/mL | 6.84 (± 3.13, min. 1.6–max. 11. 57) | 7.72 (± 4.25, min. 1.86–max. 16.85) | 8.09 (± 6.11, min. 1.39–max. 20.82) | Chi-square – 2.364 ns. |
| Leptin ng/mL | 3.78 (± 5.30, min. 0.26–max. 17.01) | 4.64 (± 5.41, min. 0.63–max. 19.35) | 4.41 (± 5.91, min. 0.13–max. 16.76) | Chi-square – 3.455 $p = 0.1$ |
| FABP4 ng/mL | 16.90 (± 6.26, min. 7.1–max. 25.12) | 16.69 (± 5.49) min. 7.58–max. 28.97) | 17.16 (± 7.54, min. 7.21–max. 35.97) | Chi-square – 2.182 ns. |

Data are presented as mean (± SD, min–max).

only fasting, but also postprandial level of FFAs was found to increase during the follow-up, which was manifested by increased plasma FFAs concentrations 2 hours after the OLTT ($p < 0.05$) (Table 4), as well as by increased AUC for FFAs changes during OLTT after 12 month of the therapy as compared with baseline (Fig. 5).

Regarding other investigated parameters (glucose, insulin, GIP), there were no statistically significant differences in oral lipid tolerance test during the 12 months of observation (Table 4). The level of TG 2 hours after the meal was higher at month 6$^{th}$ and 12$^{th}$, as compared with levels before introduction of ARV drugs ($p < 0.05$, $p < 0.01$). Postprandial response of TG, assessed as AUC increased after 6 months of the therapy, and then remained on a similar level to the end of observation (Fig. 6). Total insulin AUC was increased 6 months after the ARV drugs were introduced, and the same tendency was observed for HOMA-IR. Any significant changes (only tendency to increased release during OLTT) in GIP concentration were found in the course of ARV therapy.

Concerning proinflammatory cytokines, we observed a statistically significant decrease of the TNFα, as IL-6 plasma levels had a tendency to decrease ($p = 0.1$) (Table 2). The performed tests revealed

**Table 4.** Changes in oral lipid tolerance test (OLTT) results during 12 months of ARV therapy

| | Before introduction of ARV therapy – 0 | 6 months after introduction – I | 12 months after introduction – II | Friedman's test |
|---|---|---|---|---|
| Glucose_0 (mmol/l) | 4.83 (± 0.37, min. 4.2–max. 5.6) | 4.98 (± 0.89, min. 4.0–max. 6.2) | 4.65 (± 0.47, min. 4.3–max. 5.4) | Chi-square – 0.571 ns. |
| Glucose_2 (mmol/l) | 4.67 (± 0.65, min. 3.6–max. 6.3) | 4.75 (± 0.91, min. 3.6–max. 6.9) | 4.43 (± 0.58, min. 3.5–max. 5.6) | Chi-square – 1.273 ns. |
| Glucose_4 (mmol/l) | 4.88 (± min. 4.4–max. 6.2) | 5.23 (± 0.64, min. 4.1–max. 6.2) | 4.74 (± 0.47, min. 3.9–max. 5.6) | Chi-square – 2.279 ns. |
| Insulin_0 (μU/mL) | 9.44 (± 3.14, min. 6.1–max. 17.9) | 13.22 (± 9.16, min. 6.6–max. 39.2) | 10.24 (± 6.27, min. 5.6–max. 20.7) | Chi-square – 1.163 ns. |
| Insulin_2 (μU/mL) | 19.59 (± 8.0, min. 9.3–max. 36) | 24.91 (± 15.73, min. 7.8–max. 54.6) | 21.69 (± 7.88, min. 12.2–max. 31.8) | Chi-square – 1.273 ns. |
| Insulin_4 (μU/mL) | 10.40 (± 3.24, min. 6.3–max. 17.6) | 14.06 (± 8.49, min. 7.6–max. 31.5) | 12.24 (± 8.57, min. 12.2–max. 31.8) | Chi-square – 0.182 ns. |
| GIP_0 (pmol/L) | 80.88 (± 40.06, min. 33.65–max. 164.99) | 101.58 (± 80.24, min. 11.33–max. 342.42) | 93.78 (± 63.07, min. 34.57–max. 252.77) | Chi-square – 0.140 ns. |
| GIP_2 (pmol/L) | 491.05 (± 143.8, min. 262.23–max. 720.78) | 419.9 (± 189.6, min. 199.11–max. 934.55) | 456.37 (± 226.84, min. 183.4–max. 973.9) | Chi-square – 3.455 p = 0.17 |
| GIP_4 (pmol/L) | 264.34 (± 98.61, min. 129.82–max. 428.79) | 346.9 (± 157.8, min. 148–max. 737.87) | 268.85 (± 121.84, min. 149.99–max. 545.43) | Chi-square – 3.818 p = 0.14 |
| TG_0 (mmol/L) | 1.27 (± 0.40, min. 0.19–max. 1.4) | 1.85 (±0.68, min. 0.8–max. 3.6) | 2.34 (± 0.90, min. 1.2–max. 3.6) | Chi-square – 7.091 p < 0.05 |
| TG_2 (mmol/L) | 1.73 (± 0.43, min. 1.01–max. 2.5) | 2.42 (± 1.06, min. 1.44–max. 4.85) | 2.58 (± 1.01, min. 1.04–max. 4.22) | Chi-square – 3.818 p = 0.1 |
| TG_4 (mmol/L) | 1.88 (± 0.83, min. 0.96–max. 3.54) | 2.43 (± 1.21, min. 1.33–5.28) | 2.49 (± 1.29, min. 0.79–max. 5.42) | Chi-square – 3.818 p = 0.1 |
| FFAs_0 (mmol/l) | 0.71 (± 0.41, min. 0.19–max. 5.6) | 0.65 (± 0.31, min. 0.23–max. 1.38) | 1.0 (± 0.14, min. 0.53–max. 2.19) | Chi-square – 6.000 p < 0.05 |
| FFAs_2 (mmol/L) | 0.38 (± 0.26, min. 01–max.1.11) | 0.51 (± 0.25, min. 0.21–max. 0.99) | 0.59 (± 0.40, min. 0.24–max. 1.58) | Chi-square – 6.727 p < 0.05 |
| FFAs_4 (mmol//) | 0.59 (± 0.26, min. 0.35–max. 1.05) | 0.61(± 0.27, min. 0.23–max. 1.22) | 0.70 (± 0.38, min. 0.2–max. 1.35) | Chi-square – 4.909 p = 0.086 |

Data are presented as mean (± SD, minimum–maximum).
0 – baseline blood samples collected after 10 hours overnight fast, to assess fasting triglycerides (TG), free fatty acids (FFAs), glucose, insulin, incretin (GIP) levels.
2 – retesting the above mentioned parameters from the blood samples collected 2 hours after fatty meal ingestion. 4 – retesting the above mentioned parameters from the blood samples collected 4 hours after fatty meal ingestion.

that TNFα after the 6 months of ART was significantly lower than at the baseline ($p < 0.01$), as well as after the 12 months and baseline ($p < 0.01$). Fasting concentrations of leptin had a tendency to increase ($p = 0.1$) during the 12 months of ART (Table 2). Analysis of leptin concentrations elicited that differences in concentration were observed mainly during the first 6 months of therapy ($p = 0.09$).

We did not notice any significant changes in adiponectin or FABP4 protein during the period of the study.

# Discussion

As life of HIV infected patients is prolonged because of effective ARV therapy, the frequency of metabolic disorders is increasing and may have a significant impact on their survival [8, 11].

Taking the above into consideration we designed the follow-up study to assess the signs of metabolic disorders development, as well as inflammation connected with infection and endocrine function of adipose tissue in the group

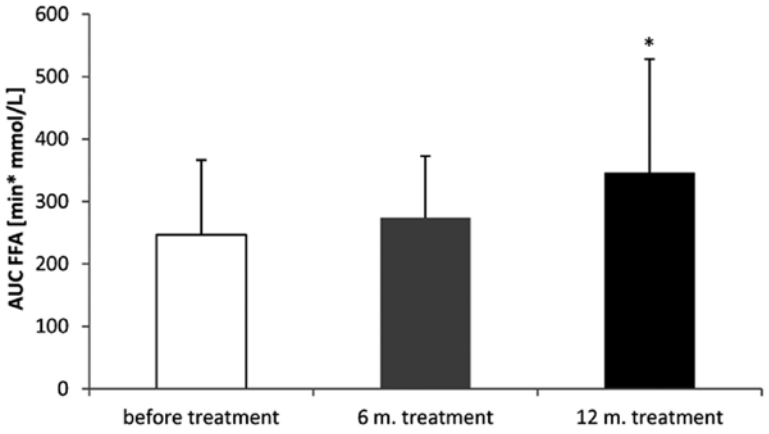


**Fig. 5.** Effect of duration of ARV PI-based therapy on postprandial free fatty acids (FFAs) concentrations. Incremental Area Under the Curves (AUCs) for FFAs during the oral lipid loading test (OLTT).

* $p < 0.05$ 12 months ARV treatment vs. before therapy

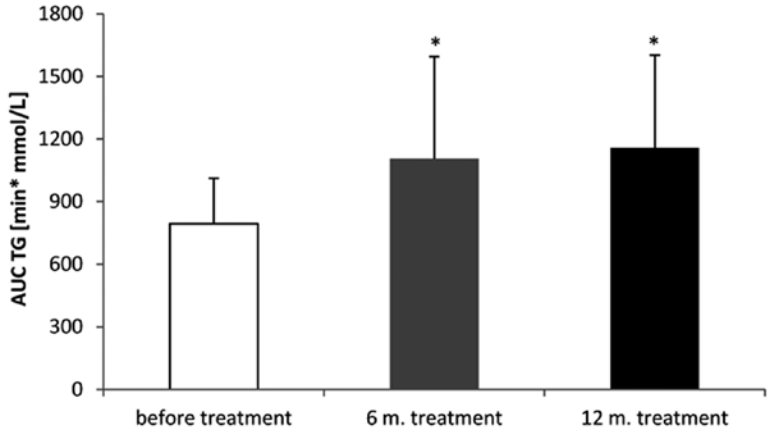


**Fig. 6.** Effect of duration of ARV PI-based therapy on postprandial triglycerides (TG) concentrations. Incremental Area Under the Curves (AUCs) for TG during the oral lipid loading test (OLLT). *$p < 0.05$ 6 months ARV treatment vs. before therapy.

* $p < 0.05$ 12 months ARV treatment vs. before therapy

of HIV infected people under the PIs-based therapy.

Conducted assessment of the body composition and fat distribution using a CT scan quantification did not reveal significant changes of adipose tissue volume during the period of 12 months as measured at abdominal and thigh level. It confirms clinical observations that modern ARV therapy less frequently causes overt lipodystrophy [11, 12]. However, in our study we observed that adipose tissue volume at thigh level measured by CT significantly increased during the first 6 months of the follow-up. Also, Calmy et al. reported the increase of the limb fat mass after 6 months of ARV therapy, although from week 24 there was a progressive loss of limb adipose tissue [13]. It must be stressed that in contrast to our protocol, in the above study patients were treated either with non-nucleoside reverse transcriptase inhibitor (NRTI) efavirenz or protease inhibitor (PI) nelfinavir, which is currently withdrawn from therapy. Also, a significant impact on their result had the fact that the backbone of ARV was the combination of NRTIs which are strongly linked with lipoatrophy development (didanosine and stavudine) [14].

In our study the adipose tissue volume at thigh level measured by CT, positively correlated with the increase of leptin concentrations. Similar observations were also previously reported in non-HIV infected obese patients [15]. Gluteofemoral adipose tissue is suggested to have special associations with metabolic health. The fat accumulation in this region can protect against insulin resistance, diabetes mellitus and cardiovascular diseases [16]. Whereas leptin is considered to be the marker of lipodystrophy in HIV infected patients [17, 18]. The relationship between the muscle fat infiltration derived from CT scans and metabolic syndrome in postmenopausal women were explored, and its correlation with the development of metabolic disturbances disorders was suggested [19]. Thus, our study, as well as the Calmy et al., can suggest that leptin and limb fat percentage independently predict the selective loss of limb fat and may occur as soon as from week 24 onwards [13]. Therefore, in spite of the lack of clinical symptoms of lipodystrophy, the changes in CT ratio of adipose tissue volume at abdomen/thigh during the follow-up point to the development of metabolic abnormalities.

ARV drugs administration resulted with the development of dyslipidemia. Triglycerides levels

significantly increased during the first 12 months, which was reported also by other investigators [20]. These observations are also supported by animal models, which proved that the administration of PI, ritonavir to mice increases levels of TG, FFAs and TC. Histopathological analysis documented that PIs-based therapy led to the inflammation of epidymal fat and increased the level of proinflammatory mediators [21].

In our study we also observed a delayed postprandial clearance of TG, which can be considered as an independent risk factor for cardiovascular diseases [22]. As adipose tissue is in large extent responsible for FFA/TG clearance on the basis of the above data, it can be concluded that this capacity of adipocytes is impaired by ARV therapy [23].

We proved that ARV therapy leads to an increase of fasting, as well as postprandial FFAs. It shows that HIV infected individuals, even without obvious lipodystrophy, experienced increased lipolysis, as well as they can have impaired adipocyte FFAs trapping. These changes in FFAs concentration can be a triggering event for the development of other metabolic complications, as hypertriglyceridemia and insulin resistance [24]. FFAs reaching the liver may up-regulate the production of TG, which will partially explain the observations mentioned above [25]. It is suggested that the oxidation rate of plasma FFAs remains unaltered and non-oxidative fatty acid disposal may be increased by reesterification in the liver [26]. Augmented intrahepatic FFAs availability contributes to the increased very low-density lipoprotein (VLDL)-triglyceride secretion rate, resulting in increase of TG concentration [26, 27]. Increased FFAs plasma concentrations lead to fat accumulation in different organs and subsequently to insulin resistance [26]. We did not observe in our study any significant increases of HOMA-IR, only a tendency, but this can be due to the short period of observation. There is also data about a potential positive impact of reducing the adipocyte size and lowering plasma FFAs. PPAR-γ, which is expressed predominantly in adipose tissue, plays crucial roles in regulating adipocyte differentiation, fatty acid metabolism. Yang et al. suggested that a reduction in adipocyte size and an increase in FFAs uptake in the adipose tissue were partly attributed to upregulation of PPAR-γ and its target genes [28]. Lack of changes in the protective adiponectin, and an increase in leptin may add to the marker of these unwanted effects. In contrast to other investigators, we did not observe changes in the concentration of FABP4 during the one year of ARV therapy, which may suggest that changes in leptin concentration is the better predictor of possible upcoming changes in fat distribution and metabolic disturbances [29]. As reported, the ARV therapy decreases the level of proinflammatory cytokines: TNFα and IL-6 at month 12 of the follow-up [4, 13].

The changes in insulin secretion was not related to the postprandial GIP secretion, since any significant changes were observed during the whole period of the study, independently if HIV infected patients were drug naive or ARV treated.

The above observations indicate that therapeutic interventions against increased blood FFAs levels can be beneficial in HIV infected ARV treated patients. It is suggested that PPAR-γ agonist rosiglitazone can decrease fasting FFAs levels in HIV individuals, although it has not been effecting postprandial FFAs concentrations [30]. Our study has some limitations connected mainly with the small sample size, so the results must be interpreted with caution. Although we have decided to publish the initial results of the presented study, as to our knowledge it is the first follow-up study on metabolic disturbances and endocrine function of adipose tissue in which patients were treated only with modern PIs with exclusion of NRTIs.

The authors have confirmed a significant impact of ARV therapy on metabolic disorders. One year ARV therapy reduces inflammation measured by TNFα and IL-6 blood concentrations. Adipose tissue remodeling associated with the increased blood concentration of FFAs, indicating lipotoxicity, as well as impaired adipocyte FFAs trapping is a triggering event in the development of hypertriglyceridemia during the course of ARV therapy. Changes in postprandial FFAs and TG indicate the increasing risk of cardiovascular diseases during the ARV therapy. We conclude also that modern ARV therapy during the period of 12 months does not induce significant changes in the total body fat content. Nevertheless fat redistribution correlated with higher leptin level, pointing to the increased risk of cardiovascular complications developing. Due to the small number of patients, results must be interpreted with caution and this suggestion needs further studies.

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## Address for correspondence:

Monika Bociąga-Jasik
Chair of Gastroenterology, Hepatology and Infectious Diseases
Department of Infectious Diseases of the Jagiellonian University
Collegium Medicum
Śniadeckich 5
31-531 Kraków
Poland
Tel.: +48 12 424 73 40
E-mail: monika.bociagajasik@gmail.com

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