

Metabolic complications and selected cytokines in HIV-infected individuals

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KEY WORDS

adipocytokines, HIV, lipodystrophy, metabolic complications

ABSTRACT

INTRODUCTION Human immunodeficiency virus (HIV)-infected individuals are at a higher risk of developing metabolic disturbances. The pathogenesis of these complications is complex and not fully explored. **OBJECTIVES** The aim of the study was to investigate the effect of HIV infection and antiretroviral (ARV) therapy on the development of metabolic changes and adipocytokine concentrations. The analysis of the differences in the investigated parameters among lipodystrophic and nonlipodystrophic patients was also performed.

PATIENTS AND METHODS A total of 42 HIV-infected patients on ARV therapy (HIV(+)ARV(+)), 13 HIV-infected ARV naive patients (HIV(+)ARV(-)), and 20 healthy controls were included in the study. A lipid profile, fasting free fatty acids (FFAs), glucose, insulin, and insulin resistance (homeostasis model assessment of insulin resistance – HOMA-IR) were tested. Serum concentrations of tumor necrosis factor α (TNF- α), interleukin 6 (IL-6), adiponectin, leptin, and fatty acid-binding protein 4 (FABP4) were determined.

RESULTS Increased FFA levels were observed in HIV(+)ARV(-) patients. HIV(+)ARV(+) patients had significantly higher triglycerides and insulin level compared with controls. HOMA-IR showed a tendency to be higher in HIV(+)ARV(+) patients compared with the other study groups. The ARV therapy longer than 2 years resulted in more pronounced metabolic abnormalities. HIV infection itself had a significant effect on inflammation expressed by elevated TNF- α and IL-6 levels. We did not observe differences in adiponectin and FABP4 concentrations among the study groups, while the leptin concentration was significantly lower in HIV-infected lipodystrophic than in nonlipodystrophic patients.

CONCLUSIONS HIV infection induces lipid disorders, especially associated with fatty acid turnover augmented by ARV therapy. Compared with FABP4, leptin is a better biological marker of metabolic complications in HIV-infected patients.

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Received: October 4, 2013.

Revision accepted:
December 3, 2013.

Published online:
December 13, 2013.

Conflict of interest: none declared.
Pol Arch Med Wewn. 2014;
124 (1-2): 27-35
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INTRODUCTION The introduction of antiretroviral (ARV) drugs has been one of the most impressive advancements in the therapy of infectious diseases since the discovery of antibiotics. The suppression of human immunodeficiency virus (HIV) replication allows to change the fatal condition into a chronic disease and significantly prolong patient survival.¹ However, HIV-infected people on highly active antiretroviral therapy (HAART) have a shorter life expectancy compared with the general population, and they are at a higher risk of developing metabolic complications such as lipodystrophy, dyslipidemia, insulin resistance, and diabetes mellitus.^{2,3}

Metabolic disorders in HIV-infected patients are connected with the side effects of HAART, but may also be linked to the lipogenic and pro-inflammatory or immunostimulatory nature of HIV infection.^{1,4} The activation of the immune system is multifactorial and is related to HIV replication as well as damage of the mucosal barriers caused by the virus and translocation of the microbial products to the circulation and damage of the thymus.¹ Different groups of ARV drugs, especially protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitors (NRTIs), but also HIV itself, are responsible for the development of lipodystrophy.^{3,5} They cause changes in

TABLE 1 Patient characteristics in the study groups

	HIV(+)ARV(+) n = 42	HIV(+)ARV(-) n = 13	HIV(-) n = 20
age, y	33.5 (24–44)	30.5 (23–42)	34 (24–44)
male, n (%)	33 (78)	11 (85)	11 (55)
female, n (%)	9 (21)	2 (15)	9 (45)
CD4, cell/ μ l	580 (152–1151)	301 (148–383)	NA
CD4, nadir cell/ μ l	193 (4–401)	274 (148–360)	NA
viral load, copii/ml	29	278 315 (5700–1,310,000)	NA
viral load during ARV therapy, copii/ml	108 531 (1852–1,370,000)	NA	NA
BMI, kg/m ²	23.5	24	24
WHR, cm	0.9 $\pm$ 0.1 (0.8–1.1)	0.9 $\pm$ 0.1 (0.8–0.1)	0.9 $\pm$ 0.1 (0.7–1.0)
body fat content, kg	16.4 $\pm$ 6.8 (2.2–42)	16.2 $\pm$ 5.1 (8.8–26.8)	18.5 $\pm$ 4.8 (10.9–31.1)
body fat content, %	21.7 $\pm$ 6.2 (5.0–40.9)	21.1 $\pm$ 6.3 (13.3–34)	25.4 $\pm$ 4.8 (14.9–34.1)
lean body mass, kg	56.7 $\pm$ 8.7 (33.1–95)	59.2 $\pm$ 5.1 (49.9–71.5)	54.3 $\pm$ 9.1 (40.2–70.6)
RMR, kcal	1664.5 $\pm$ 190.8 (1180–2372)	1587.5 $\pm$ 232.1 (1239–2161)	1751.5 $\pm$ 126.9 (1466–2089)
duration of long-term ARV therapy, y	>6 (6–10) (n = 11) >2–6 (n = 16) 2–0.5 (n = 15)	NA	NA
ARV drugs	NRTIs	abacavir/lamivudine (n = 20) tenofovir/lamivudine (n = 12) tenofovir/emtricitabine (n = 18)	NA
	PIs	atazanavir/r (n = 16)	NA
		darunavir/r (n = 11)	
		lopinavir/r (n = 10)	
		saquinavir/r (n = 5)	

Data are presented as number (percentage), number (ranges), or mean $\pm$ standard deviation (ranges).

Abbreviations: ARV – antiretroviral, BMI – body mass index, HIV – human immunodeficiency virus, NA – not applicable, NRTIs – nucleoside reverse transcriptase inhibitors, PIs – protease inhibitors, RMR – resting metabolic rate, WHR – waist-to-hip ratio

endocrine function of the adipose tissue, alter cytokine production by adipocyte and infiltrating macrophages, increase lipolysis, and activate free fatty acid (FFA) release into the circulation.^{6,7}

The role of adipokines in the pathogenesis of different metabolic complications and lipodystrophy in HIV-infected patients has been stressed in numerous studies.^{8–10} Recently, the fatty acid-binding protein 4 (FABP4) has been suggested to be another adipokine produced in adipocytes as well as macrophages and endothelial cells involved in the regulation of body-weight control, glucose, and lipid metabolism and pancreatic β -cell function.^{11–13} It has been speculated that FABP4 may participate in the pathogenesis of metabolic disturbances connected with HIV and HAART.¹⁴

The aim of the present study was to assess the effect of HIV infection and ARV therapy on the development of metabolic changes (such as dyslipidemia, insulin concentration, insulin resistance, and FFA levels) and on adipocytokine concentrations, including FABP4 in the blood. We also analyzed the differences in parameters between lipodystrophic and nonlipodystrophic patients.

PATIENTS AND METHODS HIV-infected patients from the Outpatient Clinic of the University Hospital in Kraków, Poland, were included in the study after the approval by the Ethics Committee of the Jagiellonian University Medical College (No KBET/52/B/2008). Written informed consent was obtained from all study participants. A total of 42 HIV patients on stable ARV therapy (HIV[+]ARV[+]) for at least 6 months (0.5–10 years), treated with a regimen including 2 NRTIs and 1 ritonavir-boosted PI were investigated. Detailed drug characteristics are presented in [TABLE 1](#). A total of 13 patients at the time of the recruitment into the study were ARV-naïve (HIV[+]ARV[-]). Patients with the body mass index (BMI) above 30 kg/m² as well as patients with chronic diseases, malignancy, immune-inflammatory diseases, and metabolic disorders were excluded from the study. All participants were Caucasians. The assessment of the patients' body composition including body fat content (BFC_% and BFC_{kg}), BMI, body impedance, resting metabolic rate (RMR), lean body content (LBC_{kg} and LBC_%) was done using the BF-907 Body Composition Analyser (Maltron International, United Kingdom). Anthropometric measurements including the waist-to-hip ratio (WHR) were also performed. Based on the above methods as well

TABLE 2 Metabolic abnormalities in the study groups

	HIV(+)ARV(+)	HIV(+)ARV(-)	HIV(-)	P value
TC, mmol/l	4.85 ± 1.1 (2.8–7.2)	4.04 ± 0.7 (0.7–5.1)	NA	<0.01 HIV(+)ARV(+) vs. HIV(+)ARV(-)
LDL-C, mmol/l	2.8 ± 1.0 (1.3–5.1)	2.82 ± 0.6 (0.6–3.1)	NA	<0.05
HDL-C, mmol/l	1.32 ± 0.3 (0.5–2.1)	1.14 ± 0.2 (0.8–1.4)	NA	<0.05
TG, mmol/l	1.72 ± 0.98 (0.84–6.16)	1.27 ± 0.40 (0.6–1.97)	1.0 ± 0.36 (0.53–1.62)	<0.05 HIV(+)ARV(+) vs. HIV(-)
FFAs, mmol/l	0.54 ± 0.39 (0.12–1.74)	0.71 ± 0.41 (0.19–1.4)	0.41 ± 0.17 (0.19–0.78)	<0.05 HIV(+)ARV(-) vs HIV(-)
fasting glucose, mmol/l	4.68 ± 0.67 (2.8–6.4)	4.83 ± 0.37 (4.2–5.6)	4.72 ± 0.60 (3.7–5.7)	NS
insulin, µU/ml	12.72 ± 9.07 (5.2–49.2)	9.42 ± 3.14 (6.1–17.9)	9.67 ± 2.87 (5.2–17.6)	<0.05 HIV(+)ARV(+) vs. HIV(+)ARV(-) and HIV(+)ARV(+) vs. HIV(-)
HOMA-IR	2.76 ± 2.47 (0.93–13.99)	2.03 ± 0.73 (1.27–3.90)	2.03 ± 0.73 (0.88–3.68)	0.09 (NS) HIV(+)ARV(+) vs. HIV(+)ARV(-) HIV(+)ARV(+) vs. HIV(-)

Data are presented as mean ± standard deviation (ranges).

Abbreviations: FFAs – free fatty acids, HDL-C – high-density lipoprotein cholesterol, HOMA-IR – homeostasis model assessment of insulin resistance, LDL-C – low-density lipoprotein cholesterol, NS – nonsignificant, TC – total cholesterol, TG – triglycerides, others – see [TABLE 1](#)

as the clinical assessment and self-assessment questionnaire, HIV(+)ARV(+) patients were divided into 2 groups, i.e., patients with lipodystrophy (n = 21) and those without lipodystrophy (n = 21). The control group comprised 20 healthy HIV-negative (HIV(-)), age-matched volunteers. Detailed patient characteristics are presented in [TABLE 1](#).

The CD4 cell count was analyzed by flow cytometry and HIV-1 viral load was determined by the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test (Cobas Amplicor system, Roche Diagnostics, Switzerland). Fasting serum glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were tested by routine enzymatic procedures. FFAs were measured in nonfrozen serum using an enzymatic colorimetric method (Roche Applied Science, United States). The fasting insulin level was measured by an immunoradiometric method (Diasource Immunoassays, Belgium) and read-out using a gamma counter (LKB Instruments, United States). The sensitivity of this assay was 1 µU/ml, precision within the assay was 2.1%, and inter-assay precision was 6.5%.

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated on the basis of the formula: HOMA-IR = fasting insulin (µU/ml) × fasting serum glucose (mmol/l)/22.5. A HOMA-IR above 2.5 was considered to define insulin resistance.

To assess tumor necrosis factor α (TNF-α), interleukin 6 (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 TNF-α and IL-6 were measured by high-sensitivity enzyme-linked immunosorbent assay (ELISA; R&D System, United States) according to the manufacturer's instructions with a sensitivity of 0.12 pg/ml and

0.04 pg/ml, respectively, and precision within the assay expressed as the coefficient of variation (CV%) around 6% and 7%, respectively. Interassay precision was around 10% for TNF-α and 8% for IL-6.

To assess serum leptin and adiponectin levels (human total adiponectin), immunoassays (R&D System) with a sensitivity of 7.8 pg/ml and 0.25 ng/ml, respectively, were used. CV% was 3% and 4% and interassay precision around 4% and 6% for leptin and adiponectin, respectively.

The FABP4 concentration was evaluated by the ELISA (BioVendor, Czech Republic), with a sensitivity of 0.05 ng/ml, CV% of around 2.5%, and interassay precision of around 4%.

Statistical analysis The results were shown as mean ± standard deviation. The study groups were compared using the 1-way analysis of variance. For the post hoc analysis, the Tukey test was used. For 2-group comparisons, the *t* test was used. Correlations were assessed by the Pearson test and *r* coefficients were shown. A *P* value of less than 0.05 was considered statistically significant.

RESULTS Metabolic profile Dyslipidemia was detected in 18 HIV(+)ARV(+) patients (43%). Eight patients (19%) had the serum TC level exceeding 5.2 mmol/l, 4 patients (10%) had the serum TG level exceeding 2.2 mmol/l, and 6 (14%) developed mixed dyslipidemia. In the group of HIV(+)ARV(-) patients, no changes in the lipid profile were observed. Detailed data on lipid concentrations are presented in [TABLE 2](#).

FFA concentrations were comparable in the HIV(+)ARV(+) and HIV(-) groups, but a significant difference (*P* < 0.05) was observed between the HIV(+)ARV(-) and HIV(-) groups ([TABLE 2](#)).

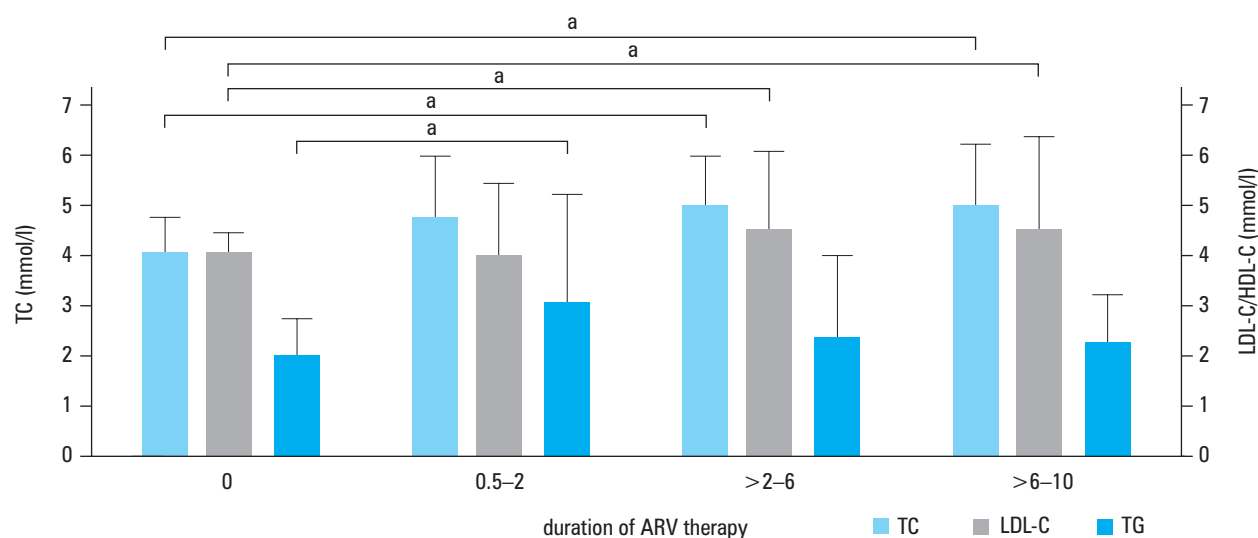


FIGURE 1 Effect of long-term antiretroviral (ARV) therapy on serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels in HIV-infected patients; data are presented as mean $\pm$ standard deviation

a $P < 0.05$

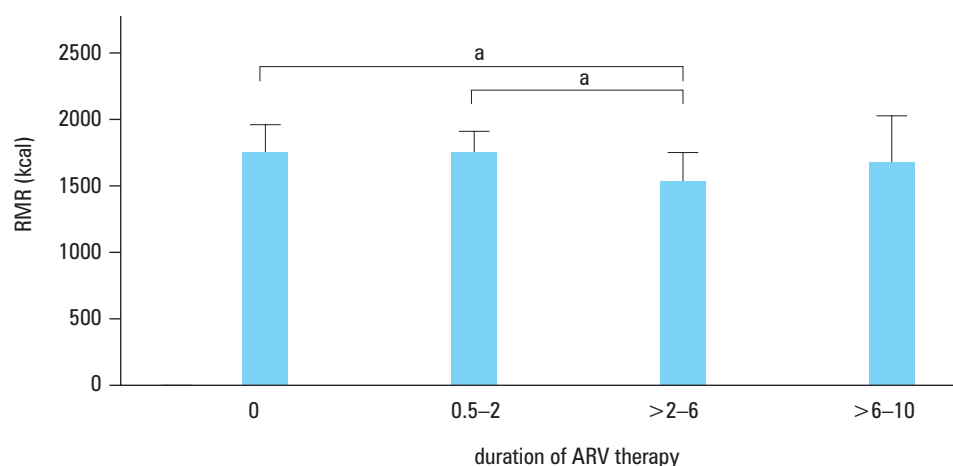


FIGURE 2 Effect of long-term antiretroviral (ARV) therapy on the resting metabolic rate (RMR) in HIV-infected patients; data are presented as mean $\pm$ standard deviation

a $P < 0.01$

There was a significant difference ($P < 0.05$) between the fasting TG level in the HIV(+)ARV(+) and HIV(-) as well as HIV(+)ARV(-) groups (TABLE 2).

The fasting glucose level exceeding 5.2 mmol/l was observed in 4 patients (9.5%), 1 patient (2%), and 1 patient (5%) in the HIV(+)ARV(+), HIV(+)ARV(-), and HIV(-) groups, respectively, while the fasting insulin level exceeding 15 μ U/ml was observed in 11 patients (26%), 1 patient (2%), and 1 patient (5%) in the HIV(+)ARV(+), HIV(+)ARV(-), and HIV(-) groups (TABLE 2).

Fourteen HIV(+)ARV(+) patients (33%) developed insulin resistance compared with 3 patients (23%) in the HIV(+)ARV(-) group, and 2 patients (10%) in the HIV(-) group (TABLE 2). There was a tendency ($P = 0.09$) for the HOMA-IR to be higher in the HIV(+)ARV(+) group compared with non-ARV-treated patients and controls. In the HIV(+)ARV(+) group, the HOMA-IR positively

correlated with the WHR ($r = 0.295$, $P < 0.05$) and fasting TG levels ($r = 0.619$, $P < 0.01$).

The duration of the ARV therapy affected the development of metabolic abnormalities. Statistically higher TC and LDL cholesterol levels were observed in patients treated with ARV drugs for more than 2 years ($P < 0.05$; FIGURE 1). However, the TG level reached the maximum values earlier during therapy and was the highest in patients treated for more than 6 months (but no longer than 2 years) (FIGURE 1). Fasting FFA levels significantly decreased in patients treated with ARV drugs for more than 2 years ($P < 0.05$), and had the lowest values in patients on HAART for more than 6 years.

The fasting insulin level and HOMA-IR had a tendency to increase during the years of ARV therapy but the differences were not statistically significant.

TABLE 3 Changes in adipocytokine concentrations in the study groups

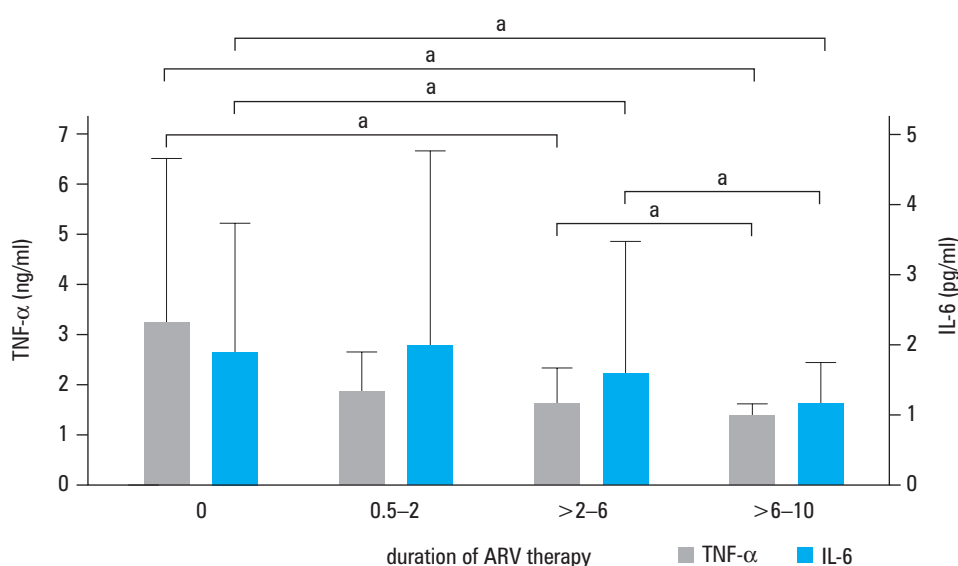
	HIV(+)ARV(+)	HIV(+)ARV(-)	HIV(-)	P value
TNF- α , pg/ml	2.10 $\pm$ 3.50 (0.92–23.85)	3.12 $\pm$ 3.26 (1.05–12.44)	1.40 $\pm$ 0.44 (0.87–2.51)	<0.05 HIV(+)ARV(-) vs. HIV(-)
IL-6, pg/ml	1.59 $\pm$ 2.01 (0.18–10.69)	1.85 $\pm$ 1.83 (0.52–7.55)	1.15 $\pm$ 1.16 (0.36–4.35)	<0.05 HIV(+)ARV(+) vs. HIV(-), <0.05 HIV(+)ARV(-) vs. HIV(-)
adiponectin, μ g/ml	6.79 $\pm$ 4.88 (1.06–18.43)	6.84 $\pm$ 3.13 (1.6–11.57)	8.16 $\pm$ 2.90 (3.14–13.22)	NS
leptin, ng/ml	3.62 $\pm$ 5.02 (0.06–24.31)	3.78 $\pm$ 5.30 (0.26–17.01)	7.32 $\pm$ 5.25 (1.32–15.95)	<0.01 HIV(+)ARV(+) vs. HIV(-) <0.05 HIV(+)ARV(-) vs. HIV(-)
leptin/adiponectin ratio	0.92 $\pm$ 1.43 (0.01–6.61)	0.67 $\pm$ 0.94 (0.03–3.22)	0.96 $\pm$ 0.76 (-0.12 to 3.20)	NS
FABP4, ng/ml	16.97 $\pm$ 8.23 (1.57–32.53)	16.90 $\pm$ 6.26 (7.1–25.12)	20.42 $\pm$ 7.22 (4.31–35.23)	NS

Data are presented as mean $\pm$ standard deviation (ranges).

Abbreviations: IL-6 – interleukin 6, FABP4 – fatty acid-binding protein 4, TNF- α – tumor necrosis factor α , others – see TABLES 1 and 2

FIGURE 3 Effect of long-term antiretroviral (ARV) therapy on serum tumor necrosis factor α (TNF- α) and interleukin 6 (IL-6) levels in HIV-infected patients; data are presented as mean $\pm$ standard deviation

a $P < 0.05$



Metabolic alterations observed during the long-term therapy were characterized by a statistically significant decrease of the lean body mass ($P < 0.01$) and RMR ($P < 0.01$) in patients treated for 2 years but no longer than 6 years (FIGURE 2). The mean serum TNF- α level was higher in the HIV(+)ARV(+) group compared with controls, although the difference was not statistically significant (TABLE 3). In contrast, HIV(+)ARV(-) patients had a statistically significantly higher TNF- α level compared with healthy individuals (TABLE 3). The mean serum IL-6 level in HIV(+)ARV(+) and HIV(+)ARV(-) patients was significantly higher compared with HIV(-) controls (TABLE 3).

The analysis of HIV(+)ARV(+) patients revealed a continuous decrease of serum TNF- α and IL-6 levels with therapy duration, and patients treated for more than 2 years had statistically lower concentrations of both cytokines ($P < 0.05$) (FIGURE 3). In the HIV(+)ARV(+) group, there was no correlation between TNF- α and IL-6 and body composition parameters. However,

the mean adiponectin level was lower in HIV-infected patients as compared with controls, but the difference was not statistically significant (TABLE 3). In patients treated for more than 2 years, a tendency of adiponectin to increase was observed although the differences were not statistically significant (FIGURE 4). In the HIV(+)ARV(+) group, adiponectin was negatively correlated with the WHR ($r = -0.371$, $P < 0.05$), BFC_{kg} ($r = -0.377$, $P < 0.01$), RMR ($r = -0.643$, $P < 0.001$), and positively correlated with HDL cholesterol levels ($r = 0.536$, $P < 0.001$). There were nonsignificant ($P < 0.1$) positive correlations with the glucose level ($r = 0.275$, $P = 0.07$) and negative with the TG level ($r = -0.281$, $P = 0.07$).

Leptin concentrations in HIV(+)ARV(+) and HIV(+)ARV(-) patients were significantly lower compared with controls (TABLE 3). The leptin-to-adiponectin ratio was the lowest in the group of HIV(+)ARV(-), but the differences were not statistically significant (TABLE 3).

TABLE 4 Adipocytokines in the group of HIV-infected patients treated with antiretroviral therapy and with or without lipodystrophy

Adipocytokines	HIV(+)ARV(+)		P value
	lipodystrophy (n = 21)	no lipodystrophy (n = 21)	
TNF- α , pg/ml	1.56 $\pm$ 0.53 (0.92–2.79)	1.41 $\pm$ 0.72 (0.97–3.87)	NS
IL-6, pg/ml	1.70 $\pm$ 2.26 (0.49–10.96)	1.13 $\pm$ 0.77 (0.27–3.24)	NS
adiponectin, μ g/ml	6.71 $\pm$ 5.15 (1.43–19.15)	6.94 $\pm$ 4.83 (1.06–19.50)	NS
leptin, ng/ml	3.0 $\pm$ 3.45 (0.06–7.4)	5.95 $\pm$ 6.35 (0.04–24.31)	<0.05
FABP4, ng/ml	15.90 $\pm$ 8.18 (3.2–30.54)	17.32 $\pm$ 7.85 (1.57–27.28)	NS

Data are presented as mean $\pm$ standard deviation (ranges).

Abbreviations: see TABLES 1 and 3

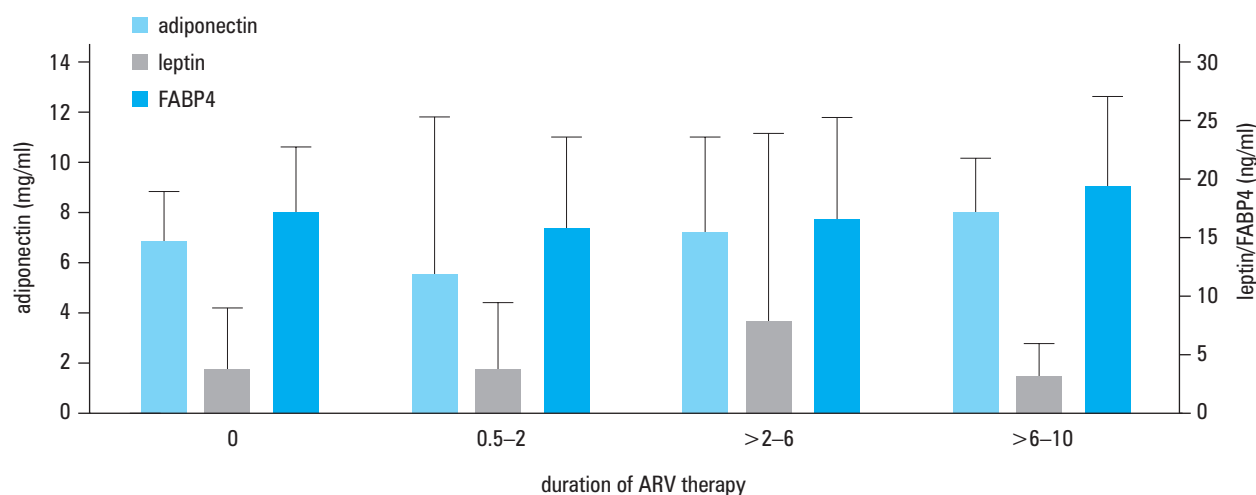


FIGURE 4 Effect of long-term antiretroviral (ARV) therapy on serum adiponectin, leptin, fatty acid-binding protein 4 (FABP4) level in HIV-infected patients; data are presented as mean $\pm$ standard deviation; lack of statistical significance

Patients treated for a period of 2 to 6 years had higher leptin levels, but then the leptin concentration decreased (FIGURE 4).

The leptin concentration in the HIV(+)ARV(+) group correlated positively with BFC (BFC_%, $r = 0.599$, $P < 0.001$; BFC_{kg}, $r = 0.575$, $P < 0.001$), BMI ($r = 0.422$, $P < 0.01$), insulin level ($r = 0.362$, $P < 0.05$), and HOMA-IR ($r = 0.310$, $P < 0.05$), as well as with IL-6 ($r = 0.451$, $P < 0.01$) and FABP4 concentrations ($r = 0.398$, $P < 0.01$).

There were no significant differences in FABP4 concentrations between the study groups (TABLE 3), but a tendency for serum FABP4 levels to decrease during the first years of ARV therapy was observed (FIGURE 4).

In the group of HIV(+)ARV(+), FABP4 correlated positively with BMI ($r = 0.575$, $P < 0.001$), BFC (BFC_%, $r = 0.621$, $P < 0.001$; BFC_{kg}, $r = 0.591$, $P < 0.001$). Correlations were also observed with glucose ($r = 0.279$, $P < 0.05$), insulin ($r = 0.277$, $P < 0.05$), and TG levels ($r = 0.3143$, $P < 0.05$).

There was no correlation between any of the investigated adipocytokines and HIV variables on enrollment into the study and on introduction of the HAART.

We also compared patients who developed lipodystrophy during the course of the ARV therapy and those without lipodystrophy. We did not observe significant differences in TNF- α , IL-6, adiponectin, and FABP4 concentrations, although the leptin concentration was significantly lower in

patients with lipodystrophy compared with those without lipodystrophy (TABLE 4). In patients with lipodystrophy, the CD4 cell count before the introduction of the ARV therapy correlated negatively with TC and LDL cholesterol levels ($r = -0.452$, $P < 0.05$ and $r = -0.479$, $P < 0.05$, respectively) and positively with the FFA level ($r = 0.680$, $P < 0.01$).

DISCUSSION Metabolic complications are increasingly common in ARV-treated patients, and they are predicted to have a significant effect on morbidity and mortality.^{3,6} Insulin resistance and impaired glucose intolerance as well as dyslipidemia seem to have a complex pathogenesis and are related to HIV itself, ARV drugs, as well as genetic predisposition.^{1,15-17}

Our results are in line with those reporting that the development of metabolic abnormalities is more commonly observed in the group of HIV-infected, ARV-treated patients.^{3,18} It should be stressed that, in contrast to other studies, we recruited only those patients who were treated with 2 NRTIs (with the exclusion of stavudine and didanosine) combined with PIs. PIs, which interact with the adipocyte proteosomal system, affect proteins involved in lipid metabolism/trafficking and cause insulin resistance.¹⁹ Our observations confirm that long-term PI-based therapy results in a significant increase of the TG level compared with ARV-naïve patients and healthy controls. As the adipose tissue is, to a large extent,

responsible for the FFA/TG clearance on the basis of the above data, it can be concluded that this capacity of adipocytes is impaired by ARV therapy.²⁰ The fasting insulin level in HIV-infected, ARV-treated patients was elevated; however, glucose concentrations were comparable between the study groups. Insulin resistance showed a tendency to be higher in ARV-treated patients compared with the control group and HIV-infected, ARV-naïve patients, indicating that the development of insulin resistance may be affected by ARV therapy. It is in contrast to a number of other investigators who reported a significant increase in the incidence of insulin resistance and diabetes in ARV-treated patients.²¹ It can be partially explained by the fact that those abnormalities are more common in ethnic minorities than in Caucasians.¹⁷

We observed a correlation between the duration of ARV therapy and the risk of metabolic disturbances. Patients who received the therapy for more than 2 years had a significant decrease of RMR. The available data on the effect of the HAART on RMR are conflicting.^{22,23} Increased RMR is positively correlated with the TNF- α concentration; therefore, a continuous decrease of the serum TNF- α level observed in the present study with therapy duration can partially explain the reported changes of RMR.²³ Also, mitochondrial dysfunction is a likely cause of an increase in RMR.²² In another study, we reported that ARV therapy ameliorated mitochondrial dysfunction.⁵

Not all metabolic disturbances can be linked directly to ARV therapy. We observed a significantly higher FFA concentration in ARV-naïve patients compared with healthy individuals. This observation indicates that the replicating HIV affects lipid metabolism (activates lipolysis of the tissue or prevents FFA deposition in the adipose tissue).²⁴ An increased serum FFA concentration is related to the degree of insulin resistance, as FFAs have been proposed to induce skeletal muscle insulin resistance.^{17,25} Our results confirm the observation that HIV itself induces lipid metabolic changes, which makes patients more vulnerable to the side effects of ARV.

Inflammation promotes insulin resistance.²⁶⁻²⁸ We demonstrated that HIV infection itself induces inflammation, which is associated with higher TNF- α and IL-6 levels, although we did not observe any correlation between those cytokines and HIV variables, which is in line with previous observations.^{6,21} IL-6 was also higher in HIV(+)/ARV(+) patients compared with controls, which indicates persistent induction of inflammation despite effective ARV therapy.

Any significant differences in adiponectin and FABP4 concentrations were observed in the study group, when the leptin level was significantly lower in both HIV(+) (treated and nontreated) patients. In the HIV(+)/ARV(+) group, leptin correlated positively with the insulin level and HOMA-IR as well IL-6 and FABP4. The leptin concentration was significantly lower

in patients with lipodystrophy as reported by other investigators.^{9,29,30}

In contrast to other studies, we did not consider FABP4 as a strong marker of lipodystrophy. However, in HIV-infected ARV-treated patients, FABP4 positively correlated with glucose, insulin, and TG concentration.³¹ FABP4 has been reported to be a marker of metabolic syndrome in the general population.^{14,31} Baseline serum FABP4 levels were considered to predict the risk for metabolic complications in HIV-infected patients.^{13,32} FABP4 also correlated with glucose, insulin, and TG concentrations in our study. The reported differences may be associated with the fact that, unlike in our study, the previous studies recruited patients with obesity and metabolic complications.³¹ Of note, in our study, patients were treated only with modern PIs, and NRTIs strongly related to lipodystrophy development were excluded.

Based on our results, we may suggest that FABP4 could be used as a marker of metabolic disturbances in HIV-infected patients, but leptin may be a more useful marker of lipodystrophy.

In conclusion, persistent inflammation is observed in HIV-infected patients, which predisposes them to the development of metabolic disturbances. HIV affects lipid metabolism inducing lipotoxicity. Persistent inflammation and lipotoxicity can promote the development of insulin resistance. Metabolic complications and their intensity depend on therapy duration. The pathophysiological mechanisms of these complications have not been explored in the present study. Leptin seems to be the best marker of lipodystrophy when FABP4 correlates with the presence of metabolic disturbances.

Acknowledgements This work was supported by a grant from Polish State for Scientific Research, Nr N N402 421 638 (granted to M.B.-J.).

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Powikłania metaboliczne i wybrane cytokiny w grupie pacjentów zakażonych wirusem HIV

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SŁOWA KLUCZOWE

adipocytokiny, HIV,
lipodystrofia,
powikłania
metaboliczne

STRESZCZENIE

WPROWADZENIE Pacjenci zakażeni wirusem HIV (*human immunodeficiency virus*) cechują się zwiększonym ryzykiem wystąpienia powikłań metabolicznych. Patogeneza tych zaburzeń jest złożona i nie do końca poznana.

CELE Celem pracy było zbadanie wpływu zakażenia HIV i terapii antyretrowirusowej (ARV) na rozwój zaburzeń metabolicznych i stężenie adipocytokin. Przeprowadzono także analizę różnic w zakresie badanych parametrów między pacjentami z lipodystrofią i bez lipodystrofii.

PACJENCI I METODY Badaniem objęto 42 pacjentów zakażonych HIV otrzymujących terapię ARV (HIV(+)ARV(+)), 13 pacjentów nieleczonych ARV (HIV(+)ARV(-)) oraz 20 zdrowych osób. Celem oceny zaburzeń metabolicznych oznaczono profil lipidowy, stężenie wolnych kwasów tłuszczowych (WKT), glukozy, insuliny oraz insulinooporność (HOMA-IR). Badano stężenie w surowicy czynnika martwicy guza (TNF-α), interleukiny 6 (IL-6), adiponektyny, leptyny i białka wiążącego kwasy tłuszczowe 4 (FABP4).

WYNIKI W grupie pacjentów HIV(+)ARV(-) obserwowano podwyższone stężenia WKT. Pacjenci HIV(+)ARV(+) mieli znacznie większe stężenia triglicerydów i insuliny w porównaniu z grupą kontrolną. Obserwowano tendencję do częstszego występowania HOMA-IR w grupie pacjentów HIV(+)ARV(+) w porównaniu do innych badanych grup. Terapia ARV prowadzona przez okres dłuższy niż 2 lata wiązała się z większym nasileniem zaburzeń metabolicznych. Samo zakażenie HIV miało wpływ na nasilenie procesu zapalnego, co wyrażało się zwiększonymi stężeniami TNF-α i IL-6. Nie stwierdzono różnic w stężeniu adiponektyny i FABP4 między badanymi grupami, natomiast stężenie leptyny było istotnie niższe w grupie pacjentów zakażonych HIV z lipodystrofią w porównaniu z grupą bez lipodystrofii.

WNIOSKI Zakażenie HIV wywołuje zaburzenia lipidowe, zwłaszcza związane ze zwiększonym obrotem kwasów tłuszczowych, które nasilają się pod wpływem terapii ARV. Leptyna jest lepszym od FABP4 biologicznym markerem rozwoju powikłań metabolicznych w grupie pacjentów zakażonych HIV.

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Praca wpłynęła: 04.10.2013.
Przyjęta do druku: 03.12.2013.
Publikacja online: 13.12.2013.
Nie zgłoszono sprzeczności
interesów.
Pol Arch Med Wewn. 2014;
124 (1-2): 27-35
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