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Associations between Hypertriglyceridemia and Serum Ghrelin, Adiponectin, and IL-18 Levels in HIV-infected Patients

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Abstract. HIV-related metabolic abnormalities include hypertriglyceridemia, hypercholesterolemia, insulin resistance, and diabetes mellitus. Recent studies suggest a role of ghrelin in promoting the deposition of triglycerides (TG) in the liver and regulating the metabolic action of adiponectin. Visceral fat is a key regulator of inflammation and it secretes proinflammatory cytokines (eg, interleukin-18, IL-18), with potential atherogenic activity. The aim of this study was to assay serum concentrations of ghrelin, adiponectin, and IL-18 in HIV+ patients, with and without hypertriglyceridemia, who were receiving highly active antiretroviral therapy (HAART). The 49 HIV+ patients were divided in 2 groups: 17 patients with serum TG concentration >200 mg/dl (group A) and 32 patients with normal serum TG concentration (group B). All subjects underwent viral and immunological evaluations and determinations of serum cholesterol, glucose, ghrelin, adiponectin, and IL-18. No differences of viral and immunological parameters were observed between the 2 groups. Serum levels of ghrelin were 768 ± 596 pg/dl in group A and 470 ± 248 pg/dl in group B ($p = 0.01$). Group A had lower serum adiponectin levels (8.4 ± 3.6 µg/dl) than group B (18.2 ± 10.1 µg/dl; $p = 0.0001$). Serum IL-18 levels were 455 ± 199 pg/ml in group A and 258 ± 233 pg/ml in group B ($p = 0.005$). The patients with hypertriglyceridemia showed a positive correlation between serum triglyceride and ghrelin levels ($r = 0.51$, $p = 0.03$). These findings suggest potential roles of ghrelin, adiponectin, and IL-18 in the pathogenesis of metabolic disorders in HIV-infected patients.

Keywords: HIV-infection, ghrelin, adiponectin, IL-18, triglycerides, hypertriglyceridemia, cytokines

Introduction

Immunity against viral infection is mediated by a combination of humoral and cellular immune mechanisms with the production of cytokines [1-3]. The interaction of the immune system with infectious organisms is a dynamic interplay of host mechanisms aimed to eliminate infections [4,5] and the pathogens' strategies are designed to permit survival despite powerful effector mechanisms [6,7]. Cytokines, as well as inhibitors affecting cytokines and chemokine receptors, have been proposed as preventive and therapeutic agents for various disorders, including cancer, autoimmunity, and viral infections. However, the clinical utility and efficacy of such agents remain to be proved.

The use of highly active antiretroviral therapy (HAART) has been shown to be effective in reducing plasma levels of HIV RNA, restoring immune functions, and increasing CD4(+) T cell counts [8]. Cytokine modulation has been observed in HAART-treated patients and their isolated lymphocytes [9,10]. However, several HAART-related adverse events have been described. The most important HAART-related adverse event is the occurrence of metabolic disorders, in particular hypertriglyceridemia, including that associated with lipodystrophic syndrome [11-15]. Several studies have investigated the potential roles of adipokines, such as ghrelin, adiponectin, and interleukin-18 (IL-18), in inducing these metabolic abnormalities [16-18].

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Ghrelin, a 28 amino acid peptide, acts as an endogenous ligand for the growth hormone (GH) secretogen receptor that is present on somatotropic pituitary gland and specific hypothalamic neuronal cells [19,20]. In humans, ghrelin is principally expressed by endocrine cells of the gastric fundus and it is involved in energy homeostasis [21]. Fasting serum ghrelin level is negatively correlated with body mass index (BMI); it is decreased in obesity and increased in cachexia [22,23]. Recently, a role of ghrelin in the regulation of lipid metabolism has been described [24]. Ghrelin enhances lipogenesis and gluconeogenesis, increases triglyceride levels, and reduces fatty acid oxidation-stimulating activity; its effect to promote triglyceride deposition is greater in liver than skeletal muscle [25].

Adiponectin is a protein produced by adipocytes that modulates insulin action and may cause loss of body weight by increasing metabolism without hunger stimulation [18]. Serum IL-18, a potent proinflammatory cytokine that has atherogenetic properties [26] through its effects on IL-6, TNF-α, and interferon-γ [27], has been found to be elevated in HIV-infected subjects [28]. Circulating levels of IL-18 are increased in obesity [29], decline with weight loss, and are elevated in HIV+ patients with lipodystrophy [30,31].

The aims of this study were two-fold: to determine serum levels of ghrelin, adiponectin, and IL-18 in HIV+ patients who were receiving HAART and to test for correlations between the circulating levels of these "adipoproteins" and the metabolic abnormalities observed in HIV infection.

Materials and Methods

Subjects. Forty-nine HIV-infected Caucasian patients (10 women, 39 men), age 44.1 ± 10 yr, were recruited from the Clinic of Infectious Diseases of University of Chieti School of Medicine. The patients had received ≥12 mo of cumulative exposure to antiretroviral regimens, including therapy with protease inhibitors (PI) and/or nucleoside reverse-transcriptase inhibitors (NRTI) and/or non-nucleoside reverse transcriptase inhibitors (NNRTI). The patients were not receiving any other therapies They were divided in two groups: 17 patients (1 woman, 16 men) with serum triglycerides (TG) levels >200 mg/dl (group A) and 32 patients (9 women, 23 men) with normal values of triglycerides (≤200 mg/dl) (group B). The hypertriglyceridemic patients had all been negative for high levels of serum tryglycerides before the onset of HIV infection, according to their clinical histories. All subjects gave written informed consent; this study was approved by the Ethics Committee of the University of Chieti School of Medicine.

Anthropometric and fat distribution indexes. All anthropometric measurements were taken in the morning by trained staff according to WHO recommendations [32]. Weight was measured to the nearest 0.1 kg and height to the nearest 0.5 cm. The body mass index (BMI) (Kg/m^2) was computed. Waist circumference, measured between the lowest rib margin and the iliac crest while the subjects were standing and breathing normally, and hip circumference, measured on the greater trochanters, were determined to calculate the waist-to-hip ratio (WHR). Bioelectric impedance analysis (BIA) was used to evaluate the percentage of fat mass (FM) and fat-free mass (FFM) (Akern Bioresearch, Florence, Italy) [33].

Blood assays. CD4- and CD8-T cell counts were obtained by cytofluorimetric assessment of lymphocyte subpopulations. Plasma viral load (HIV-RNA) was determined using the "Amplicor" method (Roche Molecular Diagnostics), with a detection limit ≤400 HIV RNA copies/ml. Fasting blood samples were drawn to assay the levels of serum glucose (glucose oxidase method), glycated haemoglobin (HPLC-Variant II, Bio-Rad Laboratories), aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides, total cholesterol, and high density lipoprotein (HDL) cholesterol (Ortho Clinical Diagnostics). LDL-cholesterol was calculated by Friedewald's formula [34]. Serum insulin levels were measured by RIA (Coat-A-Count Insulin kit, Diagnostic Products Corp.). The sensitivity, intra-assay, and inter-assay coefficients of variation of the insulin method were 1.2 μU/ml, 4.7%, and 7.3%, respectively. Insulin resistance was determined using the homeostasis model assessment index (HOMA-IR) with the following formula: {fasting insulin level (μU/ml) / fasting glucose level mmol/L} / 22.5 [35].

Serum ghrelin concentrations were assayed by an ELISA kit (Bachem Ltd.); the minumum detectable concentration was 800 – 1000 pg/ml. Serum adiponectin levels were assayed by an ELISA kit (R&D Systems); the minimum detectable concentration ranged from 7.9-8.9 μg/ml. Serum IL-18 levels were assayed by an ELISA kit (R&D Systems); the minimum detection limit estimated by serial diluition was 12.5 pg/ml, since the mean absorbance +2 SD of the 6.25 pg/ml standard was lower than the mean -2 SD of the 12.5 pg/ml standard.

Statistics. Data are reported as means ± SD. Statistical significance was assessed by the unpaired t test. A p value <0.05 was the criterion for significance. Pearson's correlation coefficients were computed for serum triglycerides levels vs serum ghrelin, adiponectin, and IL-18 levels.

Results

In patients (group A) with high serum triglycerides levels (432 ± 198 mg/dl), the HAART duration was longer than in group B (14.0 ± 3.7 yr vs 7.5 ± 3.5 yr, p = 0.0001) (Table 1). In group A, the

Table 1: Viral, immunological, and therapeutic characteristics of the study population, divided into hypertriglyceridemic subjects (group A) and normotriglyceridemic subjects (group B).

Parameter	Hypertriglyceridemic subjects (group A) (n = 17)	Normotriglyceridemic subjects (group B) (n = 32)	p
Duration HAART (years)	14.0 ± 3.7	7.5 ± 3.5	0.0001
CD4(+) (cell/µl)	509 ± 221	560 ± 301	n.s.
CD8(+) (cell/µl)	997 ± 403	931 ± 458	n.s.
Viral load positive (%)	17.6	31.3	n.s.
Viral load negative (%)	82.4	68.7	n.s.
PI (%)	35.2	34.3	n.s.
NRTI (%)	100	100	n.s.
NNRTI (%)	41.2	43.7	n.s.

PI: Protease inhibitors
NRTI: Nucleoside reverse-transcriptase inhibitors
NNRTI: Non-nucleoside reverse transcriptase inhibitors
n.s.: not significant

Table 2: Clinical characteristics of the study population, divided into hypertriglyceridemic subjects (group A) and normotriglyceridemic subjects (group B).

Parameter	Hypertriglyceridemic subjects (group A) (n = 17)	Normotriglyceridemic subjects (group B) (n = 32)	p
Age (yrs)	45.9 ± 7.8	43.2 ± 11.1	n.s.
Gender (M/F)	16/1	23/9	n.s.
Triglycerides (mg/dl)	432 ± 198	117.3 ± 33.0	0.0001
Total cholesterol (mg/dl)	198 ± 36	168.5 ± 50.9	0.04
HDL [1] (mg/dl)	31.6 ± 5.3	37.5 ± 5.5	0.0004
LDL [2] (mg/dl)	134.3 ± 28.6	112.6 ± 29.6	0.01
Fasting glucose (mg/dl)	83.9 ± 5.9	89.0 ± 13.0	n.s.
Glycated hemoglobin (%)	4.2 ± 0.9	4.6 ± 0.8	n.s.
HOMA-IR [3]	12.2 ± 1.4	12.1 ± 1.7	n.s.
AST [4] (U/L)	35.4 ± 15.7	42.8 ± 28.3	n.s.
ALT [5] (U/L)	46.0 ± 24.4	56.2 ± 42.1	n.s.
Body Mass Index (Kg/m^2)	24.4 ± 3.9	23.2 ± 3.1	n.s.
WHR [6]	0.89 ± 0.0	0.85 ± 0.0	n.s.
FM (%) [7]	22.0 ± 7.3	25.1 ± 6.8	n.s.
FFM (%) [7]	79.7 ± 6.1	76.7 ± 6.5	n.s.

[1] HDL: high density lipoprotein cholesterol
[2] LDL: low density lipoprotein cholesterol, calculated by Friedewald's formula
[3] HOMA-IR: homeostasis model assessment index for insulin resistance
[4] AST: aspartate aminotransferases
[5] ALT: alanine aminotransferase
[6] WHR: waist-to-hip ratio
7 FM and FFM: fat mass and free-fat mas calculated by bioelectric impedance analysis
n.s.: not significant

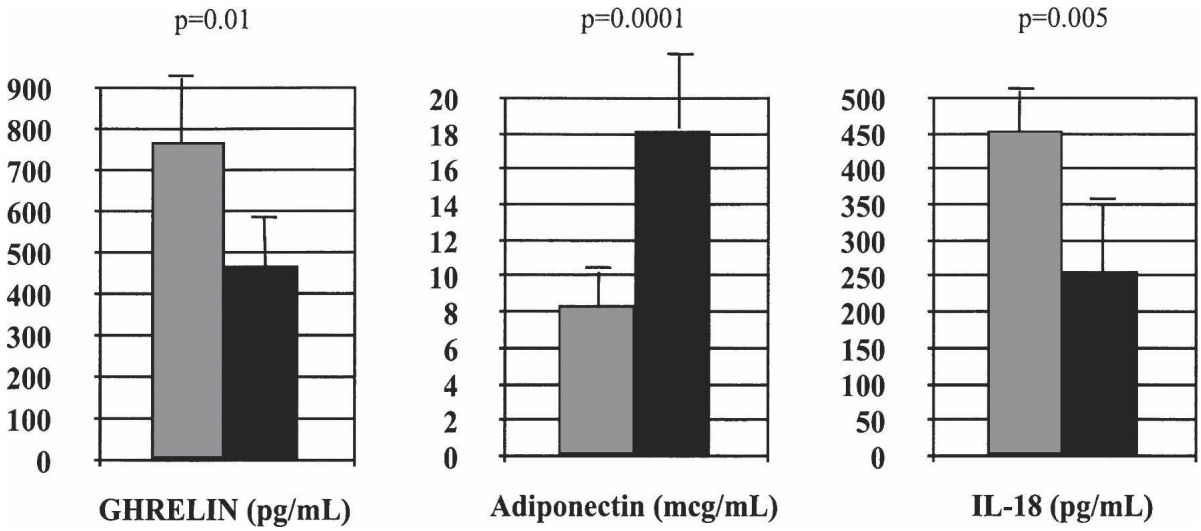

Fig. 1. Serum ghrelin, adiponectin, and IL-18 levels of the study populations. In each chart, the left (shaded) columns show the means ± SD for group A (17 HIV-infected patients with hypertriglyceridemia); the right (black) columns show the means ± SD for group B (32 HIV-infected patients with normal serum triglyceride levels).

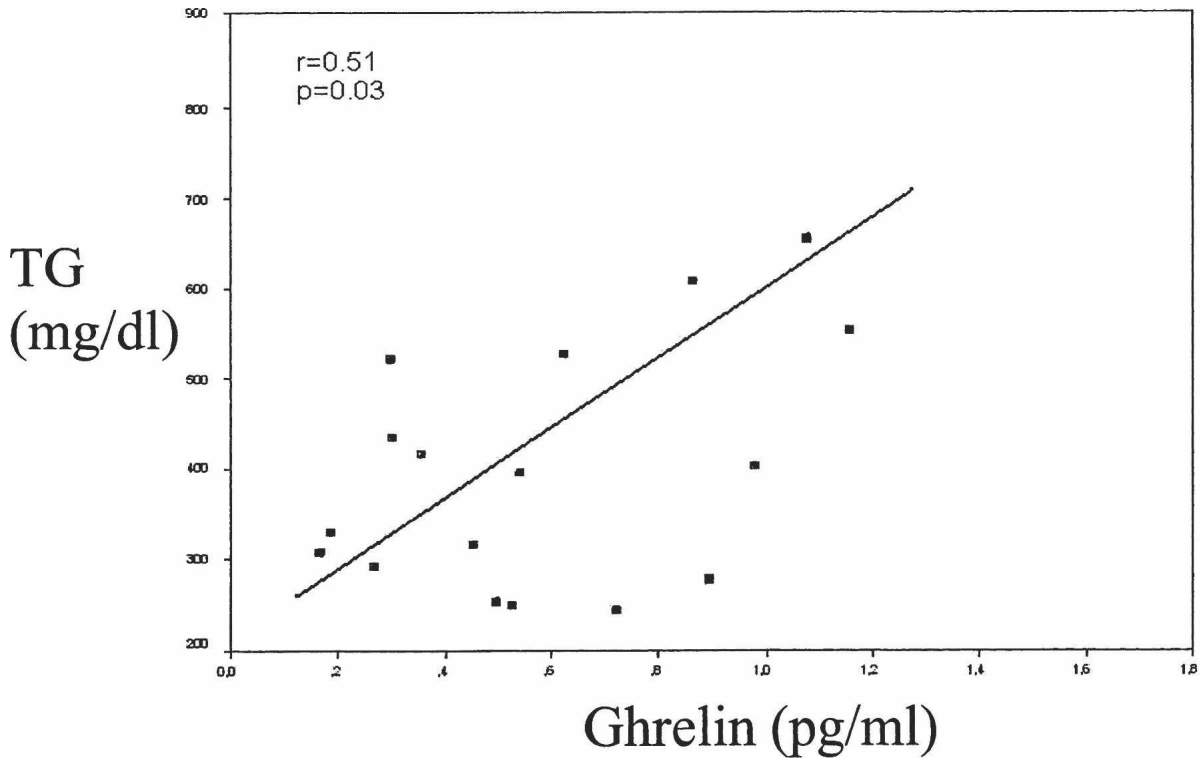

Fig. 2. The correlation of serum triglyceride (TG) and serum ghrelin levels in group A (17 HIV-infected patients with hypertriglyceridemia). Pearson's correlation coefficient (r) = 0.51 (p = 0.03).

CD4(+) T cell count was 509 ± 221 cell/µl, CD8(+) T cell count was 997 ± 403 cell/µl, and positive determination of HIV-RNA was documented only in 3 cases (17.6%) while HIV-RNA detection was negative in 14 patients (82.4%). In the patients (group B) with normal levels of serum triglycerides (117 ± 33 mg/dl), CD4(+) T cell count was 560 ± 301 cell/µl, CD8(+) T cell count was 931 ± 458 cell/µl, and positive determination of HIV-RNA was observed in 10 cases (31.3%), while HIV-RNA detection was negative in 22 patients (68.7%) (Table 1).

As shown in Table 2, the serum total cholesterol levels were 198 ± 36 mg/dL in group A and 169 ± 51 mg/dL in group B (p = 0.04). HDL cholesterol levels were lower in group A than in group B (31.6 ± 5.3 mg/dl vs 37.5 ± 5.5 mg/dl, p = 0.0004). LDL cholesterol levels in group A were 134 ± 29 mg/dl vs 113 ± 30 mg/dl in group B (p = 0.01). No significant differences were found between the two groups for fasting glucose, glycated hemoglobin, HOMA-IR, transaminase activities, or anthropometric parameters (Table 2).

Serum ghrelin levels were 768 ± 596 pg/ml in group A vs 470 ± 248 pg/ml in group B (p = 0.01). Group A showed lower adiponectin levels (8.4 ± 3.6 µg/ml) than group B (18.2 ± 10.1 µg/ml; p = 0.0001). Serum IL-18 levels were 455 ± 199 pg/ml in group A and 258 ± 233 pg/ml in group B (p = 0.005) (Fig. 1).

The patients with hypertriglyceridaemia (group A) showed positive correlation between their serum triglyceride and ghrelin levels (r = 0.51, p = 0.03), (Fig. 2). No significant correlations were observed between serum triglyceride levels and adiponectin or IL-18 concentrations.

Discussion

The principal mechanism of specific immunity against established viral infection involves cytotoxic T lymphocytes (CTLs); full differentiation of CD8(+) CTLs requires cytokines produced by several leukocytes [36,37]. Although early stages of HIV-1 infection are characterized by an intense CD8(+) T cell activation and increased expression of the chemokine receptor CCR5, essential for optimal effector function of CD8(+) T cells, these changes are associated with a poor prognosis for disease progression to AIDS in primates [38].

Highly active antiretroviral therapy (HAART) is currently the only means to halt or prevent progression of HIV infection to AIDS. Our data showed a significantly longer duration of HAART in patients with hypertriglyceridemia than in those with normal levels of serum TG. In HIV-infected patients receiving HAART, hypertriglyceridemia represents an important risk factor for cardiovascular complications and other inflammatory diseases caused by cytokines [39,40]. Several studies have reported association between hypertrygliceridemia and the HAART regimen, particularly with the use of protease inhibitors (PIs) [11,12,14]. It has been reported that PIs could interfere with the chain of free fatty acids (FFAs), inducing an increase of triglycerides. Our patients in the hypertriglyceridemic and normotriglyceridemic groups were treated with PIs and/or NRTIs and/or NNRTIs, without any significant differences of treatment modalities between the two groups (Table 1).

Brinkman et al [41] suggested that the NRTI-related syndrome may be the result of mitochondrial toxicity with impairment of hepatic glycogen and fat oxidation, leading to increased oxidation of peripheral energy stores. Other studies suggested a possible association between genetic factors and hypertriglyceridemia, and recent attention has been focused on a possible role of lipogenic cytokines [42-46]. In this context, ghrelin is a peptide involved in energy homeostasis, principally inducing food intake and GH secretion. In humans, ghrelin stimulates appetite by inducing hunger, which leads to increased food consumption and consequent metabolic alterations, with increases of body weight, body fat mass, and serum triglycerides [47]. Recently, lower levels of ghrelin have been reported in HIV+ lipodystrophic patients, compared to HIV+ patients without lipodystrophy [48]. In addition, the GH response to GHRH stimulus appears reduced because of high levels of FFAs [15]. Our HIV+ patients with hypertriglyceridaemia and increased levels of ghrelin did not show signifcantly higher values of BMI than the normal-TG group.

Barazzoni et al [25] studied in rodents the possible effect of ghrelin administration on the regulation of lipid metabolism; they described the

action of ghrelin on liver where this peptide induces an increase of triglycerides and reduces fatty acid oxidation, so that the triglycerides are deposited more in liver than in skeletal muscle [25]. Our data, in agreement with the literature, show an association between ghrelin and hypertriglyceridemia. We observed higher levels of ghrelin in patients with hypertriglyceridemia than in those with normal values of serum triglycerides, as well as positive correlation between serum ghrelin and triglyceride levels in group A.

Adiponectin, encoded by the APMI gene, is a protein expressed by adipocytes, where it acts in homeostatic control of glucose and lipid metabolism. This hormone stimulates fatty acid oxidation, reduces triglyceride levels, and increases glucose metabolism by enhancing sensitivity to insulin [49]. Adiponectin is an adipose-derived plasma protein with anti-atherogenic activities [50]. In fact, adiponectin improves dyslipidemia, decreases platelet adhesion to endothelial cells, and protects against atherosclerosis [18]. Other authors reported that hypoadiponectinemia is closely associated with the clinical phenotype of the metabolic syndrome [51]. Recent data demonstrated that adiponectin effects are mediated by its interaction with muscle and hepatic receptors through activation of AMP kinase, which in turn inhibits acetyl CoA carboxylase and increases fatty acid β-oxidation [52]. Our hypertriglyceridemic patients showed significantly lower levels of adiponectin than the normo-triglyceridemic patients. Evidence suggests that metabolic abnormalities often correlate with an inflammatory status and our hypertriglyceridemic patients showed increased levels of serum IL-18. This cytokine, through its effects on IL-6 and TNF-α, increases cardiovascular risk [29] and may play an important role in plaque destabilization and acute ischemic syndromes [53-55].

In conclusion, the adipokines may have crucial roles in the pathogenesis of hypertriglyceridaemia. Thus, monitoring of serum ghrelin, adiponectin, and IL-18 levels could be important to identify subjects with high cardiovascular risk. Our results suggest a novel paracrine pathway that operates in HIV-infection. Additional longitudinal studies are needed to confirm our findings and to find ways to prevent hypertriglyceridemia in HIV-infection.

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