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Rita Sofia Bettencourt Silva

TSH, FT3 and FT4 were not associated with changes in body composition in HIV-infected patients on combined antiretroviral therapy

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Projeto de Opção do 6º ano – DECLARAÇÃO DE REPRODUÇÃO

Nome: Rita Sofia Bettencourt Silva

Endereço eletrónico: ritabettsilva@gmail.com **Telemóvel**: +351 91 939 40 81

Número do Bilhete de Identidade: 13241843

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Faculdade de Medicina da Universidade do Porto, 21/03/2012

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DEDICATÓRIA

Aos meus pais, pelo amor incondicional, suporte emocional e liberdade para fazer as minhas escolhas;

À Beatriz, minha irmã, confidente e companheira de casa, com quem partilho diariamente todos os bons e maus momentos;

À Maria João Camões e Raquel Marçôa, pela amizade que nos une e pelo apoio prestado durante o desenvolvimento deste trabalho;

Ao meu tio Luís, pelos conhecimentos que me transmitiu e pelo seu incentivo para me superar todos os dias.

RESUMO

INTRODUÇÃO: Os doentes infetados pelo vírus da imunodeficiência adquirida (VIH) em terapêutica antirretrovírica combinada (TARC) podem ter distúrbios metabólicos, lipodistrofia e disfunção tiroideia. Tem sido investigado o impacto potencial de alterações minor da função tiroideia na composição corporal em doente eutiroideus não infetados pelo VIH, mas não na população infetada pelo VIH. Os objetivos deste estudo foram comparar os níveis de TSH, T3L e T4L nos doentes eutiroideus com e sem lipodistrofia [definida pela clínica e pela razão massa gorda (RMG)] e em quatro grupos de composição corporal; e avaliar a possível relação entre a TSH, T3L e T4L e parâmetros demográficos, antropométricos, infeciosos e metabólicos.

MÉTODOS: Estudo transversal incluindo 352 adultos caucasianos não institucionalizados infetados pelo VIH-1 sob TARC. Foram avaliados dados demográficos, caracterização da infeção VIH, função tiroideia, características antropométricas, composição corporal por DXA e parâmetros metabólicos.

RESULTADOS: Não houve diferenças significativas entre a mediana de TSH, T3L e T4L de acordo com a presença de lipodistrofia (definida pela clínica e pela RMG) e os quatro grupos de composição corporal. A TSH estava positivamente correlacionada com a idade e negativamente com a contagem de células CD4. Na análise multivariada, depois de ajustar para a idade, género e índice de massa corporal (IMC), a TSH permaneceu positivamente associada com a idade e negativamente com a contagem de células CD4. A T3L estava positivamente correlacionada com a duração da infeção por VIH e negativamente com a idade, pressão arterial sistólica (PAS) e pressão arterial diastólica (PAD). Na análise multivariada, não houve associações significativas com a T3L. A T4L estava positivamente correlacionada com a duração da infeção e negativamente com a PAD, colesterol total e C-LDL. Na análise univariada, a FT4 estava negativamente associada com o IMC, colesterol total e C-LDL, mas no modelo de regressão linear múltiplo apenas a associação com o LDL-C permaneceu significativa.

CONCLUSÕES: Não houve diferenças nos valores de TSH, T3L e T4L de acordo com a composição corporal em doentes eutiroideus infetados pelo VIH. Os níveis de TSH estavam associados positivamente com a idade e negativamente com a contagem de células CD4, mesmo na análise multivariada. Os doentes eutiroideus podem ter anomalias lipídicas e pressão sanguínea elevada.

PALAVRAS-CHAVE: VIH, terapêutica antirretrovírica, tiroide, lipodistrofia, composição corporal, razão massa gorda, pressão sanguínea, dislipidemia.

TITLE

TSH, FT3 and FT4 were not associated with changes in body composition in HIV-infected patients on combined antiretroviral therapy

Author

Rita Bettencourt Silva

Affiliation

Sixth year student of the Integrated Master Degree in Medicine, Faculty of Medicine of Porto University, Porto, Portugal

Correspondence

Rita Bettencourt Silva

Department of Endocrinology, Diabetes and Metabolism, Centro Hospitalar São João

Alameda Professor Hernâni Monteiro

4200-319 Porto

Portugal

Phone: +351 91 939 40 81

E-mail: ritabettsilva@gmail.com

ABSTRACT

BACKGROUND: Human immunodeficiency virus (HIV)-infected patients on combined antiretroviral therapy (cART) may have metabolic disorders, lipodystrophy and thyroid dysfunction. It has been investigated the potential impact of minor changes in thyroid function on body composition of euthyroid HIV non-infected patients, but not in HIV-infected population. The aims of this study were to compare TSH, FT3 and FT4 levels in euthyroid patients with and without lipodystrophy [defined by clinical and by fat mass ratio (FMR)] and in four groups of body composition; and to assess the possible relationship between TSH, FT3 and FT4 and demographics, anthropometrics, infectious and metabolic parameters.

METHODS: Cross-sectional study including 352 HIV-1-infected non-institutionalized Caucasian adults on cART. Demographic data, HIV infection characterization, thyroid function, anthropometric features, body composition by DXA and metabolic parameters were evaluated.

RESULTS: There were no significant differences between the median of TSH, FT3 and FT4 according to the presence of lipodystrophy (defined by clinical and by FMR) and the four groups of body fat composition. TSH was positively correlated with age and negatively with CD4 cell count. In multivariate analysis, after adjustment for age, gender and body mass index (BMI), TSH remained positively associated with age and negatively associated with CD4 cell count. FT3 was positively correlated with duration of HIV infection and negatively with age, systolic blood pressure (SBP) and diastolic blood pressure (DBP). In multivariate analysis, there were no significant associations with FT3. FT4 was positively correlated with duration of infection and negatively with DBP, total cholesterol and LDL-C. In univariate analysis, FT4 was negatively associated with BMI, total cholesterol and LDL-C, however in the multiple linear regression model (with age, gender and BMI as covariates) only the association with LDL-C remained significant.

CONCLUSIONS: There were no differences in TSH, FT4 and FT3 values according to body composition in euthyroid HIV-infected patients. TSH levels were positively associated with age and negatively with CD4 cell count, even in multivariate analysis. Euthyroid patients can have lipid abnormalities and raised blood pressure.

KEYWORDS: HIV, antiretroviral therapy, thyroid, lipodystrophy, body composition, fat mass ratio, blood pressure, dyslipidemia.

BACKGROUND

The acquired immune deficiency syndrome (AIDS), primarily reported in 1981, became a leading cause of mortality worldwide [1]. With the successful introduction of combined antiretroviral therapy (cART) in 1996, human immunodeficiency virus (HIV)-related morbidity and mortality have declined substantially [1, 2]. However, and due to an increased survival, there are more and more people living with this infection. At the end of 2010 it was estimated that nearly 34 million adults and children were living with HIV infection worldwide [2].

The use of cART has been associated with many side effects, including lipodystrophy, dyslipidemia, insulin resistance, increased blood pressure, decreased bone mineral density and dysfunction of the adrenal, gonadal and thyroidal axes [3-5]. Önen suggested that these comorbidities are not part of the "normal" aging process [4]. However, the mechanisms responsible for cART-related metabolic disorders are not fully understood and there is a growing recognition that inflammatory cytokines and HIV infection by itself can be the etiologic agents of these metabolic abnormalities [5, 6].

Lipodystrophy is characterized by selective loss of adipose tissue from particular anatomical regions and can be localized or generalized [7]. Acquired lipodystrophies occur more frequently than the inherited types and the most prevalent subtype in the clinic is acquired partial lipodystrophy related to cART in HIV-infected patients [7, 8]. With the beginning of molecular genetic era, congenital and familial types were associated with mutant gene products and not only characterized by clinical features. However, the mechanisms responsible for the relationship between HIV and lipodystrophy remain partially unknown [7]. HIV-associated lipodystrophy syndrome is characterized by loss of subcutaneous fat (lipoatrophy) from the face and limbs with or without deposition of excess fat (lipohypertrophy) in the neck and upper back (causing a double chin and a buffalo hump), abdomen and trunk, resulting in peripheral fat wasting and central adiposity [7-9]. Some patients have peripheral lipoatrophy, others have isolated abdominal prominence and a substantial portion has mixed forms including both phenotypes [9]. HIV-associated lipodystrophy has been linked to components of metabolic syndrome (MS), such as high blood pressure, dyslipidemia and insulin resistance, leading to an increased cardiovascular disease risk [3, 7]. The relationship between cART and fat redistribution is associated with prolonged duration of treatment and is highly linked to the use of protease inhibitors (PI) drugs,

although it has been also related to other antiretroviral drugs [3, 8, 9]. In his clinical review, Chen reported that prevalence of lipodystrophy among HIV-infected patients was highly variable according to different authors, ranging between 8% and 84% (due to different definition and diagnosis criteria). He noticed that near 40% of patients treated with PI developed lipodystrophy [8].

Thyroid hormones regulate the metabolism and function of many tissues, such as liver, heart, skin, muscle and adipose tissue. They are involved via thyroid hormone receptors (TRs) in adipogenesis and adipose tissue lipogenesis and lipolysis [10, 11]. All the isoforms of TRs (both TRA1 and TRB1) are expressed in white adipose tissue [12]. Moreover, the monocarboxylate transporter 8 (MCT8) is a thyroid-hormone-specific transporter that is ubiquitously expressed, including in human subcutaneous adipose tissue (SAT) [12]. Overt hypothyroidism is clearly associated with body weight excess and obesity, but the potential impact of minor changes in thyroid function on anthropometric measures of euthyroid HIV non-infected patients remains under investigation [13-16]. So far there are still no studies exploring this relationship among HIV-infected patients.

Due to the important role of TH on adipocyte metabolism and as HIV-associated lipodystrophy results from changes in adipose tissue, we hypothesized that minor changes in thyrotropin (TSH), free 3,5,3'-triiodothyronine (FT3) and free thyroxine (FT4) may have a significant role in the peculiar body fat redistribution of these patients.

The aims of the current study were (1) to compare the levels of TSH, FT3 and FT4 in euthyroid patients with and without lipodystrophy [(defined by clinical and by fat mass ratio (FMR)] and in four different groups of body composition (no lipodystrophy; isolated central fat accumulation; isolated lipoatrophy; and mixed forms of lipodystrophy); (2) and to assess the possible relationship between TSH, FT3 and FT4 and demographic, anthropometric, infectious and metabolic parameters.

METHODS

Subjects and study design

As part of a cross-sectional study, 352 clinically stable HIV-infected non-institutionalized Caucasian adults (234 men and 118 women) receiving antiretroviral therapy, consecutively referred from the Infectious Diseases Outpatient Clinic were evaluated. Only patients on cART were included, because lipodystrophy is related with this therapy [8]. Patients without thyroid function test results and with thyroid dysfunction (such as overt and subclinical hypothyroidism, overt and subclinical hyperthyroidism, Graves' disease, Hashimoto thyroiditis, papillary thyroid carcinoma, toxic multinodular goiter, isolated low FT4 level and nonthyroidal illness syndrome) were excluded. The study protocol was approved by the Hospital Ethics Committee and each patient provided informed consent.

Clinical assessments

For each patient the following information was collected using a standardized protocol: demographic data (age, gender, smoking status), history of hypertension, diabetes and use of antihypertensive, antidiabetic and lipid-lowering drugs, duration of HIV infection, HIV infection risk factors, duration of cART and characterization of the infection. The "Centers for Disease Control and Prevention" (CDC) criteria for staging of HIV infection were used [17]. The HIV infection risk factors were classified into four different groups: intravenous drug use; homosexual and bisexual contact; heterosexual contact; and other (hemophiliacs, transfused patients and unknown risk). Antiretroviral treatment analysis included only the last therapy used up to collecting data, the type of drugs used and the total duration of cART since infection diagnoses.

Weight, height, resting blood pressure, circumferences of neck, waist, hip, thigh and arm were measured, as previously described [18, 19]. The subjects were standing upright, with the face directed forwards, arms by the sides and gluteal muscles and shoulders relaxed. All measurements were taken by the same well-experienced physician with standard techniques [20]. Body weight was measured using a TANITA scale (Tanita®, model TBF 300), with patients wearing light clothes without shoes. Height was measured to the nearest centimetre in the standing position using a wall stadiometer (Holtain Limited

Crymych, Dyfed®). Body mass index (BMI) was calculated as weight in kilograms divided by the squared height in metres. Resting blood pressure was measured on a single occasion using a standard mercury sphygmomanometer with the cuff on the left upper arm.

Clinical lipodystrophy (CL) was defined as a peripheral lipoatrophy with or without a central fat accumulation assessed by both patient and practitioner [21]. Patients were classified as without CL when none of the previously described features were present. Presence of central fat accumulation or abdominal prominence was defined by the measurement of waist circumference using the International Diabetes Federation (IDF) criteria for MS, which recommends that the threshold for abdominal obesity must be ≥ 94 centimetres in men and ≥ 80 centimetres in women [22]. The clinical assessment was performed by the same practitioner. As previously described [21], the patients were placed into four different categories according to the presence or absence of either lipoatrophy or abdominal prominence: 1) No lipodystrophy – patients without clinical lipoatrophy and without abdominal prominence; 2) Isolated central fat accumulation – patients without clinical lipoatrophy and with abdominal prominence; 3) Isolated lipoatrophy – patients with clinical lipoatrophy and without abdominal prominence; 4) Mixed forms of lipodystrophy – patients with clinical lipoatrophy and with abdominal prominence.

Body composition was assessed with whole-body dual-energy X-ray absorptiometry (DXA – Lunar Expert XL). DXA measurement was performed while the patient was in a supine position, with standard positioning of the arms and feet. Markers used in this study for trunk and lower limbs that defined regions of interest were those indicated by the manufacturer. Regional fat mass values were grouped and analyzed for the following anatomical regions: arms, legs, trunk and total body. The FMR was calculated as the ratio between the percentage of the trunk fat mass and the percentage of the lower limbs fat mass (FMR = % of the trunk fat mass/ % of the lower limb fat mass) [23, 24].

Laboratory analysis

A venous blood sample was drawn after a 12-hour overnight fast. All the samples were analyzed at the central laboratory of our hospital. Plasma glucose, total cholesterol, low density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were determined using automatic standard routine enzymatic methods [18]. CD4 cell count was determined by flow cytometry and plasma HIV-1 RNA loads were measured by a quantitative reverse transcriptase

polymerase chain reaction, with a lower limit of detection of 50 copies/mL. Leptin (human leptin RIA, kit, Linco Research) and adiponectin (human adiponectin RIA, kit, Linco Research) were performed in Nobre Laboratory of the Porto Medical School. The intra-assay and inter-assay precision was 4.6% and 5.0%, respectively, for leptin; the intra-assay and inter-assay precision was 1.78% and 9.25%, respectively, for adiponectin. The MS was defined using the most recent criteria for its clinical diagnosis, proposed by several major organizations (including IDF) in 2009 [22].

TSH, FT3 and FT4 serum levels were measured by a chemiluminescence method (Abbott kit). The normal serum levels for TSH, FT4 and FT3 were defined according with the reference values of the central laboratory of our hospital. The normal range levels were 0.350 to 5.500 mUI/mL for TSH, 0.89 to 1.80 ng/dL for FT4 and 2.3 to 4.2 pg/mL for FT3. Euthyroidism was defined as normal TSH, T3L and FT4 values. The diagnosis of thyroid disease was made from laboratory parameters, clinic database and thyroid medication use.

Statistical analysis

Data were described as mean and standard deviation (SD) for quantitative variables or as median and respective interquartile range (IQR) and compared using the Student-t test or the Mann-Whitney test, respectively. For the comparison between the four groups of fat distribution and the thyroid function parameters the Kruskall-Wallis test was used. Categorical variables were described as counts and proportions, and compared using the chi-square or Fisher's exact test.

For estimating the association between thyroid function parameters (TSH, FT3, FT4) levels and anthropometric, metabolic and body composition characteristics, Spearman correlation coefficients were calculated.

As the dependent variables (TSH, FT3, FT4) had distributions different than the normal, they were log transformed. Using the log transformed dependent variables; multivariate linear regression models were computed to estimate the independent association between thyroid function parameters and metabolic and clinical characteristics.

Statistical analysis was performed using the SPSS version 19.0 software (SPSS Inc., Chicago, Illinois, USA). All probabilities were two tailed and p values <0.05 were regarded as significant.

RESULTS

This study included 352 (234 men and 118 women) euthyroid HIV-infected patients on cART. Table 1 shows the demographic, anthropometric, infectious and cardiovascular risk factors of the sample according to the four groups of body fat distribution. Men had more frequently isolated lipoatrophy or absence of lipodystrophy while women had isolated central fat accumulation and mixed forms of lipodystrophy. Mixed forms were more prevalent in older patients. Regarding smoking status, current smokers were more frequently classified as with no lipodystrophy and with isolated lipoatrophy, while isolated central adiposity and mixed forms were more frequent in those patients that never smoked.

Regarding anthropometric parameters, as expected patients with abdominal prominence (both isolated and mixed forms) had a higher mean of BMI, neck, waist, hip, thigh and arm circumferences and waist/hip ratio. On the other hand, FMR mean was higher in patients with isolated lipoatrophy or mixed forms. The prevalence of isolated lipoatrophy and mixed forms of lipodystrophy were higher in patients with longer duration of HIV infection and cART. As far as HIV risk factors are concerned, patients with isolated lipoatrophy were more frequently intravenous drug users while those into other body composition group had usually heterosexual risk. The prevalence of PI therapy was higher in patients without lipodystrophy or with isolated central fat accumulation. No statistically significant differences were found between the four groups of body composition and viral suppression, CD4 cell count, CDC stage and use of nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI).

Considering metabolic parameters and cardiovascular risk factors, SBP was lower in patients with isolated lipoatrophy, serum glucose was higher when mixed forms were present, total cholesterol and LDL-C levels were significantly higher in patients with isolated central fat accumulation and mixed forms. No differences were observed regarding to DBP, HDL-C and TG. Leptin mean levels were higher in patients with central fat accumulation and mixed forms, while the adiponectin levels were significantly higher in patients without lipodystrophy.

Regarding thyroid function, there were no statistically significant differences between the median of TSH, FT3 and FT4 according to the presence of lipodystrophy defined clinically or by the FMR (Table 2) and the four groups of body fat composition (Table 3). Also, the median of TSH, FT3

and FT4 did not differ significantly according to MS features (Table 4), BMI categories and presence or absence of hypertension (data not shown).

TSH levels were positively correlated with age ($r = 0.134$; $p = 0.013$) and negatively correlated with CD4 cell count ($r = -0.138$; $p = 0.010$) (data not shown). In multivariate analysis, after adjustment for age, gender and BMI, TSH levels remained positively associated with age ($\beta = 0.007$; $p = 0.005$) and negatively associated with CD4 cell count ($\beta = -0.107$; $p = 0.006$) (Table 5).

FT3 levels were positively correlated with duration of HIV infection ($r = 0.109$; $p = 0.048$) and negatively with age ($r = -0.121$; $p = 0.027$), SBP ($r = -0.162$; $p = 0.003$) and DBP ($r = -0.119$; $p = 0.031$) (data not shown). On the other hand, although FT3 levels were negatively associated with gender in univariate analysis ($\beta = -0.002$; $p = 0.001$), after adjustment no significant associations remained (Table 6).

FT4 levels were positively correlated with duration of HIV infection ($r = 0.109$; $p = 0.046$) and negatively with DBP ($r = -0.121$; $p = 0.026$), total cholesterol ($r = -0.142$; $p = 0.009$) and LDL-C ($r = -0.177$; $p = 0.001$) (data not shown). In univariate analysis, FT4 levels were negatively associated BMI ($\beta = -0.008$; $p = 0.035$), total cholesterol ($\beta = -0.001$; $p = 0.018$) and LDL-C levels ($\beta = -0.001$; $p = 0.002$) and tended to be positively associated with duration of HIV infection ($\beta = 0.052$; $p = 0.059$). However in the multiple linear regression model (with age, gender and BMI as covariates) only the association between FT4 and LDL-C remained statistically significant ($\beta = -0.001$; $p = 0.006$) (Table 7).

Interestingly, there was no correlation between TSH, FT3 and FT4 levels (data not shown). Also, there were no statistically significant correlations between TSH, FT3 and FT4 and BMI, cART duration, waist, hip and thigh circumferences, waist/hip ratio, glucose, HDL-C, TG, leptin, adiponectin and fat mass (free, total, upper limbs, lower limbs and trunk) (data not shown).

DISCUSSION

Among HIV-infected patients, some studies have reported that overt thyroid dysfunction occurs between 1% and 2% and subclinical abnormalities may be presented in nearly 35% (often subclinical hypothyroidism) [6, 25, 26].

Studies on the relationship of thyroid function and lipodystrophy are scarce [6, 27, 28]. Some authors described that lipodystrophy wasn't associated with thyroid function abnormalities [6, 27]. Indeed, to knowledge of the authors, this was the first study carried out in HIV-infected patients that investigated the possible relationship between changes of thyroid function at the normal range and lipodystrophy. In our study, there weren't statistically significant differences in TSH, FT3 and FT4 levels between patients with and without lipodystrophy (defined by clinical and by FMR) and in four different groups of body composition. Furthermore, no statistically significant correlation was found between thyroid measurements and anthropometric (BMI, waist, hip and thigh circumferences and waist/hip ratio) or body composition parameters (free, total, upper limbs, lower limbs and trunk fat mass).

Studying lipodystrophy remains difficult because there is a lack of well-defined diagnostic criteria, differences among sample populations and a poor knowledge of all the underlying pathogenic mechanisms [8]. Despite our results, particular observations reported on literature support the hypothesis that changes of thyroid function may be associated with HIV-related lipodystrophy and that this relationship may not be observed by serum thyroid hormone measurements but only in target cell or at the molecular level (probably with differences between lipoatrophic and lipohypertrophic areas). First, HIV infection itself has been associated with higher resting energy expenditure (REE) and cART with decreased REE [29, 30]. Sutinen showed that lipodystrophy by itself was associated with increased REE, since REE was significantly higher in HIV-patients on cART with lipodystrophy compared with those without lipodystrophy, even when adjusted for body weight and fat free mass [31]. Lipodystrophic patients had a higher caloric intake, which can be regulated by thyroid hormones [31]. It was described a negative correlation between REE and TSH levels and an association of minor changes in thyroid function with significant changes in REE [32]. So hypermetabolic state in lipodystrophy may be due to thyroid overactivity or in the upper-normal limit. Finally, a recent study involved patients with familial

partial lipodystrophy (Dunnigan variety) reported that thigh SAT from lipodystrophic patients had lower MCT8 and higher type 2 iodothyronine deiodinase (DIO2) mRNA expression than thigh and abdominal SAT from controls. Then, it was suggested that changes in local thyroid hormones availability occur in lipoatrophic areas [28].

Interestingly in our sample there was no statistically significant correlation between TSH and thyroid hormone levels (both FT3 and FT4). The individual changes on feedback mechanism of pituitary-thyroid axis and the pulsatile TSH secretion pattern can explain this finding [33]. Furthermore, it was suggested that genetic changes can affect the thyrotrophs sensitivity to the feedback regulation by thyroid hormones [34]. Therefore, FT4 and especially FT3 (the active cellular form of thyroid hormone) values may better reflect the physiological actions of thyroid function in different tissues.

TSH levels were positively correlated with age, while FT3 levels were negatively correlated with age. In multivariate analysis, there was a statistically significant positive association between TSH and age, regardless the gender and BMI. In previous studies involving both HIV-infected and uninfected populations, the results are controversial. Different authors had reported an inverse association between age and TSH [35], a positive association in both genders [14, 36] and also a positive correlation between TSH and age only in women [33].

In our euthyroid sample, it was found a statistically significant negative correlation between TSH (but not thyroid hormones) and CD4 cell count. Also in multivariate analysis, after adjustment for age, gender and BMI, TSH levels remained negatively associated with CD4 cell count. This relationship with immune system was also described by other authors in HIV-infected population. Madeddu observed a negative correlation between TSH and CD4 cells nadir in HIV-infected patients on cART [26]. On the other hand, Collazos found a positive correlation between FT4 and CD4 cell count [37] and Beltran showed that low CD4 cell count was a risk factor for hypothyroidism [6]. This relationship is likely to be mediated by proinflammatory cytokines as tumor necrosis factor alpha (TNF-α), which has been found increased in HIV patients both before and during therapy and has receptors in thyroid follicular cells [26, 38]. In our study, FT3 and FT4 levels had a statistically significant positive correlation with the duration of HIV infection. However, in univariate and multivariate analysis, there was a lack of association between there parameters. There were no associations of thyroid function

regarding to the cART duration, which is not concordant with the positive correlation between TSH value and therapy duration described by Madeddu [26].

The influence of thyroid function on blood pressure homeostasis has been evaluated during the last years. Both overt and subclinical hypothyroidism are often associated with arterial hypertension (from 10 to 25% has diastolic hypertension), due to an increased systemic vascular resistance, increased arterial stiffness and changes in sodium homeostasis [39-41]. On the other hand, hyperthyroidism is a secondary cause of isolated systolic hypertension because FT3 reduces peripheral vascular resistance, increases heart rate and consequently raises cardiac output, leading to a widened pulse pressure [39, 40]. Even in euthyroid patients, thyroid function has been associated with blood pressure homeostasis [41-45], but unlike this sample previous studies only included HIV non-infected patients.

In our sample, TSH, FT3 and FT4 levels didn't differ significantly according to the presence of hypertension (only 38.2% had hypertension), but FT3 levels tended to be lower among hypertensive patients [$p = 0.051$ (data not shown)]. Some authors showed that euthyroid hypertensive patients had higher TSH levels [41-43]. Gumieniak reported that lower FT4 index (an estimative for FT4 values, resulting from the product of T4 total by the thyroid hormone binding ratio) and higher TSH levels within the normal range were linked to hypertension, independently of age, gender, BMI, race, smoking status and insulin sensibility. In his study, the lower FT4 index was an independent predictor of blood pressure salt-sensitivity, one of the key elements in the pathogenesis of hypertension [41].

In our study, it was only able to establish statistically significant correlations between thyroid hormones (but not TSH) and blood pressure. Serum FT3 was negatively correlated with both SBP and DBP and serum FT4 was negatively correlated only with DBP. It has been showed that FT3 and FT4 are direct vasodilator agents that promote relaxation of vascular smooth-muscle cells and of skeletal muscle resistance arterioles, with consequent decrease of systemic vascular resistance [40, 46], which justifies these results. However, in multiple linear regression model (with age, gender and BMI as covariates) there were no associations between FT3, FT4 and blood pressure. It was an expected result because age and BMI are positively associated with blood pressure [43].

Some studies have showed a positive association between TSH within the reference range and SBP and DBP, considering both hypertensive and normotensive subjects [42-44]. Iqbal observed a linear rise in DBP and also in SBP (but only in females) with the increase of normal range TSH quartile,

independently of age, BMI and smoking status [43]. In a cross-sectional study, Saltiki reported a positive correlation between TSH and DPB (not SBP), but not in multivariate analysis [44]. On the other hand, controversial studies didn't show relationship between blood pressure and thyroid function at the normal range [47, 48]. Gumieniak observed an excessive aggregation of high-normal TSH values in hypertensive families and a higher serum TSH levels in healthy subjects with family history of hypertension compared with those with negative family history. He suggested the existence of genetic variants affecting both blood pressure regulation and TSH levels [45].

Looking for BMI, patients with isolated central fat accumulation and with mixed forms of lipodystrophy had a significant higher mean of BMI. As expected, leptin mean levels were higher in patients with central fat accumulation and mixed forms, since leptin concentration is proportional to adipocyte mass [49]. In our sample, thyroid function into normal range didn't differ according to BMI and there was no significant correlation between these two parameters. It was only observed a negative association between BMI and FT4 in univariate (but not multivariate) analysis. In euthyroid HIV non-infected patients, previous studies found an association between BMI and changes in thyroid function, but this relationship could differ between lower grades of overweight and morbid obesity. Asvold e Nyrnes reported a positive association between BMI and TSH among men and women [13, 14], while Makepeace showed a significant inverse relationship between FT4 concentration and BMI in euthyroid subjects [15]. Michalaki showed that euthyroid morbidity obese subjects have raised T3, FT3, T4 and TSH levels compared with those not overweight [16]. This relationship seems to be changed by smoking status, but it is still controversial [13-15]. Indeed, some studies didn't found any association between thyroid function and BMI [34, 50]. The relationship between obesity and lipodystrophy in HIV-infected patients was already studied previously [19].

In our population, FT4 levels were negatively correlated with total cholesterol and LDL-C. In univariate analysis there was also found a positive association between FT4 levels and total cholesterol and LDL-C. However in the multiple linear regression model (with age, gender and BMI as covariates) only the association between FT4 and LDL-C remained statistically significant. There was no association between thyroid function and HDL-C and TG. Overt and subclinical hypothyroidism is associated with dyslipidemia [39]. According to previous studies including HIV non-infected patients, TSH within the reference range were positively associated with total cholesterol and LDL-C and

negatively with HDL-C [51]. Indeed, a large population-based study including 30000 subjects found that TSH levels were positively correlated with total cholesterol, LDL-C and TG and negatively correlated with HDL-C [52]. This association could be modified by the age, insulin sensitivity, smoking status and time since the last meal [51, 52].

As mentioned previously, this was the first study performed on HIV-infected patients to investigate the association between lipodystrophy and thyroid function at the normal range. One of the strengths of our study was the absence of inter-observer variability because all measurements were taken by the same practitioner. Also there was a careful adjustment for the main confounding factors as age, gender and BMI. However the present study has some limitations. There is a possible selection bias since all patients included were referred by the Infectious Disease specialist specifically for lipodystrophy or metabolic disorders related to cART. It cannot be discarded the influence of pre-HIV body composition, the cumulative exposure of each drug and the nadir value of CD4 cells, factors that could contribute to the risk of lipoatrophy or abdominal prominence that weren't evaluated. Due to a cross-sectional study, it was only able to establish associations and correlations, but not determine causality or risk factors between thyroid function and other variables. Finally, the studied sample was relatively small, there was homogeneity of age strata compared to other studies (only 15.5% of our sample were 60 years or older) and a lack of comparison with a seronegative control group. Therefore, further prospective larger studies are needed, starting before cART and with a controlled follow-up, to find additional associations and to determine causality between thyroid function and metabolic disorders related to cART.

CONCLUSION

TSH levels were positively associated with age and negatively with CD4 cell count, even in multivariate analysis. Despite of exclusion of patients with thyroid abnormalities from analysis, these results raised the interesting possibility that even in the normal range of thyroid function patients can have lipid abnormalities and raised blood pressure. So, thyroid function must be considered as a continuum spectrum from the euthyroid status to subclinical and overt thyroid dysfunction.

Our data suggest that thyroid function doesn't seem to be involved in body fat redistribution among HIV-infected patients at least at global serum level, but we do not discard that changes in local thyroid hormones availability occur in lipoatrophic or lipohypertrophic areas. However further studies are required at this issue.

LIST OF ABBREVIATIONS

AIDS - acquired immune deficiency syndrome;

BMI - body mass index;

cART - combined antiretroviral therapy;

CDC - Centers for Disease Control and Prevention;

CL - clinical lipodystrophy;

DIO2 - type 2 iodothyronine deiodinase;

DXA - dual-energy X-ray absorptiometry;

FMR - fat mass ratio;

FT3 - free 3,5,3'-triiodothyronine;

FT4 - free thyroxine;

HIV - human immunodeficiency virus;

IDF - International Diabetes Federation;

IQR - interquartile range;

LDL-C - low density lipoprotein cholesterol;

HDL-C - high-density lipoprotein cholesterol;

MCT8 - monocarboxylate transporter 8;

MS - metabolic syndrome;

NRTI - nucleoside reverse transcriptase inhibitors;

NNRTI - non-nucleoside reverse transcriptase inhibitors;

PI - protease inhibitors;

REE - resting energy expenditure;

SAT - subcutaneous adipose tissue;

SD - standard deviation;

TG - triglycerides;

TNF-α - tumor necrosis factor alpha;

TRs - thyroid hormone receptors;

TSH - thyrotropin.

COMPETING INTERESTS

The author declares that she has no competing interests.

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Table 1: Demographic and clinical characteristics according to four groups of body fat distribution.

	No lipodystrophy	Isolated central fat accumulation	Isolated lipoatrophy	Mixed forms of lipodystrophy	P
N (%)	42 (13.8)	85 (27.9)	95 (31.1)	83 (27.2)	
Demographics					
Gender [n (%)]					
Male	35 (83.3)	40 (47.1)	84 (88.4)	48 (57.8)	
Female	7 (16.7)	45 (52.9)	11 (11.6)	35 (42.2)	<0.001
Age [years, median (IQR)]	40.0 (16.0)	42.0 (18.5)	42.0 (10.0)	51.0 (17.0)	<0.001
Smoking history [n (%)]					
Never	13 (31.7)	44 (51.8)	21 (22.8)	41 (50.0)	
Current	24 (58.5)	25 (29.4)	58 (63.0)	21 (25.6)	
Former	4 (9.8)	16 (18.8)	13 (14.1)	20 (24.4)	<0.001
Anthropometrics					
Weight [kg, mean (SD)]	64.8 (8.3)	79.0 (14.3)	61.5 (9.6)	71.1 (13.1)	<0.001
Height [m, mean (SD)]	1.67 (0.10)	1.64 (0.09)	1.67 (0.08)	1.64 (0.10)	0.005
BMI [kg/m^2, mean (SD)]	23.0 (2.3)	29.5 (4.6)	21.9 (2.8)	26.5 (3.5)	<0.001
Hip circumference [cm, mean (SD)]	93.8 (4.3)	104.3 (8.5)	88.6 (5.1)	94.5 (6.0)	<0.001
Waist circumference [cm, mean (SD)]	84.1 (6.7)	102.1 (10.9)	83.0 (8.9)	92.4 (12.1)	<0.001
Thigh circumference [cm, mean (SD)]	46.8 (4.1)	52.0 (5.3)	44.8 (3.7)	47.4 (7.8)	<0.001
Neck circumference [cm, mean (SD)]	36.4 (3.4)	37.6 (3.8)	36.1 (3.2)	38.2 (4.2)	0.004
Arm circumference [cm, mean (SD)]	26.0 (2.9)	29.0 (2.8)	25.5. (2.6)	28.0 (4.8)	<0.001
Waist/hip ratio [mean (SD)]	0.90 (0.08)	0.98 (0.08)	0.94 (0.06)	1.03 (0.07)	<0.001
FMR by DXA [median (IQR)]	0.95 (0.78)	1.17 (0.52)	1.76 (1.36)	1.91 (1.39)	<0.001
Infectious					
HIV-infection [years, median (IQR)]	7.0 (5.0)	6.0 (6.0)	9.0 (5.0)	9.0 (5.0)	<0.001
HIV RNA (<50) [n (%)]					
Yes	32 (80.0)	69 (84.1)	86 (90.5)	75 (90.4)	
No	8 (20.0)	13 (15.9)	9 (9.5)	8 (9.6))	0.235
CD4 cell count (cells/mm^3)					

< 200 [n(%)]	5 (11.9)	4 (4.7)	8 (8.4)	7 (8.4)	
≥200 [n(%)]	37 (88.1)	81 (95.3)	87 (91.6)	76 (91.6)	0.505
CDC stage [n (%)]					
A	20 (47.6)	50 (58.8)	52 (55.3)	46 (55.4)	
B	3 (7.1)	1 (1.2)	0 (0.0)	0 (0.0)	
C	19 (45.2)	34 (40.0)	42 (44.7)	37 (44.6)	0.129
cART [years, median (IQR)]	4.0 (6.0)	4.0 (5.0)	8.0 (5.0)	8.0 (5.0)	<0.001
IP [n (%)]	25 (62.5)	51 (62.2)	52 (55.3)	38 (45.8)	0.029
NNRTI [n (%)]	18 (45.0)	35 (42.7)	47 (50.0)	45 (54.2)	0.169
NRTI [n (%)]	37 (92.5)	78 (95.1)	93 (98.9)	82 (98.8)	0.118
HIV risk factor [n (%)]					
Intravenous drug use	15 (35.7)	14 (16.7)	43 (45.7)	10 (12.0)	
Homosexual contact	5 (11.9)	4 (4.8)	12 (12.8)	11 (13.3)	
Heterosexual contact	22 (52.4)	66 (78.6)	33 (35.1)	59 (71.1)	
Other	0 (0.0)	0 (0.0)	6 (6.4)	3 (3.6)	<0.001
Cardiovascular Risk Factors					
SBP [mmHg, median (IQR)]	120.0 (20.0)	120.0 (30.0)	110.0 (20.0)	120.0 (20.0)	0.003
DBP [mmHg, median (IQR)]	80.0 (10.0)	80.0 (10.0)	80.0 (10.0)	80.0 (10.0)	0.733
Glucose [mg/dL, median (IQR)]	90.0 (23.0)	92.0 (23.0)	93.0 (27.0)	99.0 (39.0)	0.023
Total cholesterol [mg/dL, median (IQR)]	216.0 (60.0)	235.7 (58.9)	212.6 (56.8)	229.7 (73.0)	0.030
HDL-C [mg/dL, median (IQR)]	44.9 (12.0)	48.0 (13.8)	43.2 (13.1)	46.5 (11.9)	0.085
LDL-C [mg/dL, median (IQR)]	130.0 (48.6)	139.7 (48.0)	120.4 (45.3)	137.6 (51.1)	0.032
TG [mg/dL, median (IQR)]	213.0 (173.0)	178.0 (266.0)	231.0 (250.0)	242.0 (178.0)	0.234
Leptin [ng/mL, mean (SD)]	3.00 (3.72)	10.25 (9.91)	2.00 (1.78)	4.35 (4.97)	<0.001
Adiponectin [ng/mL, mean (SD)]	4735.5 (5865.6)	1898.0 (2595.0)	2990.0 (7539.2)	4338.4 (3829.5)	0.025

Table 2: Thyroid function according to the presence of lipodystrophy (clinically and FMR defined).

	Lipodystrophy		
	Clinical		
	Yes	No	p
TSH [mUI/mL, median (IQR)]	1.59 (1.08)	1.44 (1.18)	0.113
FT3 [pg/mL, median (IQR)]	2.98 (0.71)	2.97 (0.73)	0.792
FT4 [ng/dL, median (IQR)]	0.94 (0.20)	0.96 (0.20)	0.671
	FMR		
	Yes	No	p
TSH [mUI/mL, median (IQR)]	1.53 (1.13)	1.55 (1.18)	0.437
FT3 [pg/mL, median (IQR)]	2.98 (0.73)	2.88 (0.76)	0.491
FT4 [ng/dL, median (IQR)]	0.92 (0.21)	0.92 (0.19)	0.864

Table 3: Thyroid function according to the four groups of body fat distribution.

	Lipodystrophy classified by the four groups of body fat distribution				
	No lipodystrophy	Isolated central fat accumulation	Isolated lipoatrophy	Mixed forms of lipodystrophy	p
TSH [mUI/mL, median (IQR)]	1.40 (1.09)	1.52 (1.50)	1.51 (0.94)	1.71 (1.21)	0.294
FT3 [pg/mL, median (IQR)]	2.97 (0.76)	2.98 (0.75)	3.07 (0.73)	2.84 (0.67)	0.126
FT4 [ng/dL, median (IQR)]	0.96 (0.16)	0.94 (0.84)	0.97 (0.24)	0.91 (0.18)	0.223

Table 4: Median levels of each parameter of thyroid function according to the presence of metabolic syndrome and its individual features.

	TSH [mUI/mL, median (IQR)]		FT3 [pg/mL, median (IQR)]		FT4 [ng/dL, median (IQR)]	
High blood pressure (mmHg)						
Absent	1.53 (1.21)		3.05 (0.68)		0.95 (0.20)	
Present	1.53 (1.10)		2.85 (0.69)		0.92 (0.19)	
P		0.885		0.030		0.389
High fasting glucose (mg/dL)						
Absent	1.65 (1.36)		2.97 (0.61)		0.96 (0.24)	
Present	1.51 (1.08)		3.01 (0.81)		0.94 (0.19)	
P		0.108		0.429		0.154
Low HDL-C (mg/dL)						
Absent	1.52 (1.20)		3.00 (0.69)		0.95 (0.18)	
Present	1.60 (1.15)		2.96 (0.75)		0.94 (0.21)	
P		0.696		0.177		0.669
High TG (mg/dL)						
Absent	1.49 (1.22)		2.97 (0.69)		0.96 (0.19)	
Present	1.57 (1.15)		3.00 (0.74)		0.94 (0.20)	
P		0.613		0.788		0.680
Waist circumference (cm)						
Absent	1.54 (1.12)		3.04 (0.76)		0.96 (0.19)	
Present	1.50 (1.20)		2.93 (0.71)		0.92 (0.20)	
P		0.942		0.131		0.429
Metabolic syndrome						
Absent	1.52 (1.25)		3.00 (0.68)		0.96 (0.21)	
Present	1.56 (1.09)		2.96 (0.80)		0.92 (0.20)	
P		0.845		0.270		0.092

Table 5: Association between TSH levels and demographic and clinical characteristics.

	β	p	Adjusted β*	p
TSH (mUI/mL)				
Age (years)	0.006	0.008	0.007	0.005
Gender	-0.020	0.726	0.0004	0.994
BMI (kg/m^2)	-0.005	0.391	-0.005	0.408
CD4 ($cells/mm^3$)	-0.108	0.005	-0.107	0.006

*-The coefficients were adjusted for age, gender and BMI.

Table 6: Association between FT3 levels and demographic and clinical characteristics.

	β	p	Adjusted β*	p
FT3 (pg/mL)				
Age (years)	-0.022	0.424	-0.002	0.162
Gender	-0.002	0.001	-0.016	0.572
BMI (kg/m^2)	-0.003	0.269	-0.002	0.395
SBP (mmHg)	-0.001	0.074	-0.001	0.325
DBP (mmHg)	-0.001	0.103	-0.001	0.200
Duration of infection (years)	0.024	0.229	0.020	0.330

*-The coefficients were adjusted for age, gender and BMI.

Table 7: Association between FT4 levels and demographic and clinical characteristics.

	β	P	Adjusted β*	p
FT4 (ng/dL)				
Age (years)	-0.001	0.575	-0.001	0.745
Gender	-0.062	0.096	-0.059	0.122
BMI (kg/m^2)	-0.008	0.035	-0.007	0.057
DBP (mmHg)	-0.002	0.158	-0.002	0.258
Total cholesterol (mg/dL)	-0.001	0.018	-0.001	0.064
LDL-C (mg/dL)	-0.001	0.002	-0.001	0.006
Duration of infection (years)	0.052	0.059	0.047	0.093

*-The coefficients were adjusted for age, gender and BMI.

Anexo I – Normas editoriais da Revista "BMC Infectious Diseases"

BMC Infectious Diseases
Instructions for authors – Research article

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- Abstract
- Keywords
- Background
- Methods
- Results and discussion
- Conclusions
- List of abbreviations used (if any)
- Competing interests
- Authors' contributions
- Authors' information
- Acknowledgements
- Endnotes
- References
- Illustrations and figures (if any)
- Tables and captions
- Preparing additional files

The **Accession Numbers** of any nucleic acid sequences, protein sequences or atomic coordinates cited in the manuscript should be provided, in square brackets and include the corresponding database name; for example,

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The Abstract of the manuscript should not exceed 350 words and must be structured into separate sections: **Background**, the context and purpose of the study; **Methods**, how the study was performed and statistical tests used; **Results**, the main findings; **Conclusions**, brief summary and potential implications. Please minimize the use of abbreviations and do not cite references in the abstract. **Trial registration**, if your Research article articles reports the results of a controlled health care intervention, please list your trial registry, along with the unique identifying number (e.g.**Trial registration**: Current Controlled Trials ISRCTN73824458). Please note that there should be no space between the letters and numbers of your trial registration number. We recommend manuscripts that report randomized controlled trials follow the CONSORT extension for abstracts.

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Kharitonov SA, Barnes PJ: **Clinical aspects of exhaled nitric oxide.** *Eur Respir J*, in press.

Published abstract

Zvaifler NJ, Burger JA, Marinova-Mutafchieva L, Taylor P, Maini RN: **Mesenchymal cells, stromal derived factor-1 and rheumatoid arthritis [abstract].** *Arthritis Rheum* 1999, **42:**s250.

Article within conference proceedings

Jones X: **Zeolites and synthetic mechanisms.** In *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Edited by Smith Y. Stoneham: Butterworth-Heinemann; 1996:16-27.

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Whole issue of journal

Ponder B, Johnston S, Chodosh L (Eds): **Innovative oncology.** In *Breast Cancer Res* 1998, **10:**1-72.

Whole conference proceedings

Smith Y (Ed): *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Stoneham: Butterworth-Heinemann; 1996.

Complete book

Margulis L: *Origin of Eukaryotic Cells.* New Haven: Yale University Press; 1970.

Monograph or book in a series

Hunninghake GW, Gadek JE: **The alveolar macrophage.** In *Cultured Human Cells and Tissues.*Edited by Harris TJR. New York: Academic Press; 1995:54-56. [Stoner G (Series Editor): *Methods and Perspectives in Cell Biology*, vol 1.]

Book with institutional author

Advisory Committee on Genetic Modification: *Annual Report.* London; 1999.

PhD thesis

Kohavi R: **Wrappers for performance enhancement and oblivious decision graphs.** *PhD thesis.* Stanford University, Computer Science Department; 1995.

Link / URL

The Mouse Tumor Biology Database [http://tumor.informatics.jax.org/mtbwi/index.do]

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Zheng, L-Y; Guo, X-S; He, B; Sun, L-J; Peng, Y; Dong, S-S; Liu, T-F; Jiang, S; Ramachandran, S; Liu, C-M; Jing, H-C (2011): Genome data from sweet and grain sorghum (Sorghum bicolor). *GigaScience*.http://dx.doi.org/10.5524/100012.

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  - SWF (Shockwave Flash)
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- Use hard returns only to end headings and paragraphs, not to rearrange lines.
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