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Diferenças entre géneros na relação entre os androgénios e a distribuição de gordura corporal no doente com infeção VIH / Gender different associations of androgens to body fat mass distribution in HIV infected patients

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FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO
UNIDADE CURRICULAR PROJETO DE OPÇÃO DISSERTAÇÃO / MONOGRAFIA

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NOME

Maria Leonor Pires da Silva

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Gender different associations of androgens to body fat mass distribution in HIV infected patients

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Gender different associations of androgens to body fat mass distribution in HIV infected patients

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Abstract:

Background: Little is known about the influence of sex hormones levels on the development of lipodystrophy, but it affects body fat distribution in healthy men and women. The aim of this study was to evaluate the relationship between lipodystrophy defined by the Fat Mass Ratio (FMR) and sex hormones in HIV-infected patients under combined antiretroviral therapy.

Methods: Cross-sectional study included 208 patients with HIV-infection under combined antiretroviral therapy, 136 men and 72 women. Body composition was evaluated by whole-body dual-energy X-ray absorptiometry and total, visceral (VAT), and subcutaneous adipose tissue (SAT) by tomography scanner. Hormonal analyses included Total and free testosterone, dehydroepiandrostenedione-sulfate (DHEA-S), androstenedione, sex hormone-binding globulin (SHBG) and oestradiol.

Results: Lipodystrophy was present in 29.2% of women and 47.1% of men. SHBG in lipodystrophic men and oestradiol in lipodystrophic women were lower. Total fat, SAT and homeostatic model assessment of insulin resistance (HOMA-IR) were higher in patients with isolated central fat accumulation. VAT and VAT/SAT ratio were higher in mixed forms of lipodstrophy. Women had lower oestradiol values in isolated central fat accumulation and mixed forms. Women showed a positive correlation between total abdominal fat and free testosterone and VAT with free testosterone. Negative correlations were seen between total abdominal fat and SHBG, SAT and SHBG, VAT and oestradiol and VAT/SAT ratio and oestradiol. Men had negative correlation between total abdominal fat and total testosterone, free testosterone, androstenedione, SHBG and oestradiol, SAT and total testosterone, free testosterone, androstenedione and SHBG, VAT and total testosterone, SHBG and oestradiol, and VAT/SAT with DHEA-S and SHBG.

Conclusions: Body fat distribution in HIV patients under combined antiretroviral therapy seems to be, at least in part, related with sex hormones and different associations were observed in both genders. Lower oestradiol values in women and lower SHBG values in men are related with presence of lipodystrophy especially with central fat accumulation. Total testosterone seems to be important to lipodystrophy phenotype in men.

Key words: HIV infection, lipodystrophy, gender, androgens, oestradiol, insulin resistance

Background:

Changes in body fat are common in human immunodeficiency virus (HIV) positive patients under antiretroviral therapy (cART) [1-3]. The prevalence of this modification varies from 10% to 85%, depending on the type of cART and the criteria used in different studies [4].

The pathophysiology of lipodystrophy is multifactorial and not be completely understood [5-8]. Little is known about the influence of sex hormone levels on the development of lipodystrophy. Nevertheless, sex hormones affect body fat distribution in healthy men and women [5, 9, 10].

Despite the contradictory results in studies with HIV-infected patients, androgen deficiency in HIV young men is not unusual (20-30%) with testosterone levels starting to decline at younger ages [11]. HIV lipodystrophic men could present normal testosterone levels but those without lipodystrophy shows higher testosterone levels [5]. Normal dehydroepiandrostenedione-sulfate (DHEA-S) was reported in lipodystrophic patients [5, 12]. Oestradiol may be decreased in HIV lipodystrophic men[5] with alterations in sex hormone-binding globulin (SHBG) levels remaining controversial[11]. Insulin resistance may affect the circulating sex hormones in HIV lipodystrophic men[5].

There are few studies about those alterations in women. Nevertheless, hyperandrogenemia in HIV lipodystrophic women with an increased free testosterone (fT) level but normal DHEA-S levels was described [13]. SHBG seems to be increased in HIV women, returning to normal in lipodystrophic patients[13]. The reduced androgens levels have been associated with increased body mass index (BMI) [14]. Low total testosterone (TT) are responsible for many metabolic changes that may result in a visceral fat accumulation [5, 9]. However, there is no consensus as some older studies showed a correlation of androgen deficiency with weight loss [12].

The aim of this study was to evaluate the relationship between lipodystrophy defined by the Fat Mass Ratio (FMR) and sex hormones (TT, fT, DHEA-S, androstenedione, SHBG and oestradiol) in HIV-infected patients under cART.

Methods:

This cross-sectional study included 208 adult patients with serologically documented HIV infection on cART, 136 men and 72 women. All patients were referred from the Infectious Diseases Department to the Endocrinology Outpatient Clinic of Hospital São João (a tertiary centre in Northern Portugal) due to lipodystrophy or any metabolic disorders.

Patients with acute or severe illness, previous treatment with androgen-derived agents and concurrent endocrinopathy (e.g. uncontrolled hypothyroidism, adrenal insufficiency, hyperprolactinemia, known pituitary disease) were excluded.

The study protocol was approved by the Hospital Ethics Committee for Health and all patients provided informed consent.

Clinical assessment

For each patient, the following information was collected using a standardized protocol: demographic data (age, gender), smoking history (current, past, or never) and alcohol consumption (yes or no), duration of HIV infection, HIV infection risk factors, type and duration of cART, viral load and CD4 cells count.

HIV stage was defined using the “Centers for Disease Control and Prevention” (CDC) criteria considering 3 clinic stages: asymptomatic (A), symptomatic without Acquired ImmunoDeficiency Syndrome (AIDS)-defining illness (B) and AIDS-defining illness (C)[15].

Body weight was measured using TANITA (Tanita®, model TBF 300) scale and height was measured to the nearest centimetre in the standing position using a wall stadiometer (Holtain Limited Crymych, Dyfed®). Circumferences of neck, waist, hip, thigh and arm were measured, as previously described [16, 17] .

BMI was calculated as weight divided by height squared (kg/m2). Clinical lipodystrophy was defined as a peripheral lipoatrophy with or without central fat accumulation assessed by both patient and practitioner [18]. Based on the body composition evaluated by whole-body dual-energy X-ray absorptiometry (DEXA) and total, visceral, and peripheral fat, evaluated by tomography scanner, as described in detail in a previous study [18]. The presence of lipodystrophy was based FMR, calculated as described in other study [18]. The patients were classified into 4 different groups according to the presence or absence of clinical lipoatrophy and abdominal prominence (central obesity: waist circumference >102 cm in men and >88cm in women, as defined by NCEP-ATP III modified criteria for metabolic syndrome[19]): group 1 - No lipodystrophy (without clinical lipoatrophy and abdominal prominence); group 2 - Isolated central fat accumulation (abdominal prominence without clinical lipoatrophy); group 3 - Isolated lipoatrophy (clinical lipoatrophy without abdominal prominence); group 4 - Mixed forms of lipodystrophy (clinical lipoatrophy and abdominal prominence) [17]. The clinical assessment was performed by the same practitioner.

Laboratory analysis

An early morning venous blood sample was drawn after a 12-hour overnight fast. All samples were analysed at the central laboratory of our hospital. Fasting glucose and insulin were

determined. Insulin resistance was defined by the homeostasis model assessment of insulin resistance (HOMA-IR) and insulin sensitivity by the quantitative insulin sensitivity check index (QUICKI) and Matsuda index [20]. TT and SHBG were determined using Cobas e411 (*Roche Diagnostic)*. fT levels were calculated according to Vermeulen formula. Androstenedione levels were obtained by chemiluminescence immunoassay using IMMULITE 2000 from Siemens. It was performed an electrochemiluminescence immunoassay to evaluate oestradiol and DHEA-S levels using Cobas e411 from Roche.

The CD4+ cell count ($\times 10^6$ cell/L) was assessed by flow cytometry and plasma HIV viral load by quantitative reverse transcriptase polymerase chain reaction (*Roche Diagnostic*).

Statistical analysis

Statistical analysis was performed using SPSS version 22.0 software (SPSS Inc., Chicago, Illinois, USA). Categorical variables are presented as absolute frequencies and proportions, and were analysed using Chi-square or Fisher's exact test. Continuous variables are presented as means and standard deviations or, in case of skewed distributions, as medians and interquartile ranges and were analysed using the Student's t-test and Mann-Whitney test appropriate. The Kolmogorov-Smirnov test was used to test the normality of distribution for each quantitative variable. All probabilities were two tailed and p values <0.05 were considered to be statistically significant. Spearman's correlation coefficient was used to assess the correlation between variables.

Results:

A total of 208 HIV infected patients under cART were included. Seventy-two (35%) were women, 29.2% with lipodystrophy and 136 (75%) were men, 47.1% with lipodystrophy (Table 1).

Men with lipodystrophy were older than those without lipodystrophy but no age difference was observed in women. Patients with lipodystrophy had higher CD4 count. Lipodystrophic groups were associated with a longer duration of cART.

No significant differences were found, concerning HIV infection duration, different HIV stages, smoking history and alcohol consumption in both groups.

In respect of anthropometric measures, hip circumference mean was smaller in lipodystrophic women, but this difference was not observed in males. VAT median levels were higher in men and women with lipodystrophy, but SAT median levels were only lower in men with lipodystrophy. VAT/SAT ratio was higher in both lipodystrophic groups.

No differences regarding waist, thigh and arm circumference and total fat were observed when these groups were compared.

Hormonal analysis showed significantly lower median SHBG levels in men. Women with lipodystrophy presented significant lower median oestradiol values.

HOMA-IR mean level was higher in patients with lipodystrophy.

Analysing the 4 different body composition distribution groups in female (Table2), 44.4% presented isolated central fat accumulation, 13.9% presented isolated lipoatrophy and 36.1% presented mixed forms of lipodystrophy. No significant differences were observed in HIV infection duration. Longer cART duration was observed in isolated lipoatrophy and in mixed forms of lipodystrophy. High waist, arm and hip circumferences were found in patients with isolated central fat accumulation and mixed forms of lipodystrophy, and high thigh circumference in those with isolated central fat accumulation. Total fat and SAT were higher in patients with isolated central fat accumulation. VAT and VAT/SAT ratio were higher in mixed forms of lipodystrophy. No significant

differences regarding HOMA-IR score were observed in female. Regarding hormonal values in women, oestradiol was lower in those with isolated central fat accumulation and mixed forms of lipodystrophy. In women younger than 50 years of age, those with isolated central fat accumulation and mixed forms of lipodystrophy had lower oestradiol levels, but no difference was observed in those with more than 50 years (but a small number was included).

In the male group, 23% presented isolated central fat accumulation, 45.6% presented isolated lipoatrophy and 25% presented mixed forms of lipodystrophy (Table 3). Isolated lipoatrophy was associated with longer HIV infection duration. Longer cART duration was observed in isolated lipoatrophy and in mixed forms of lipodystrophy. High waist, arm and hip circumferences were found in patients with isolated central fat accumulation and mixed forms of lipodystrophy, and high thigh circumference in those with isolated central fat accumulation. Total fat and SAT were higher in patients with isolated central fat accumulation. VAT and VAT/SAT ratio were higher in mixed forms of lipodystrophy. Higher HOMA-IR score was found in those isolated central fat accumulation and mixed forms of lipodystrophy. Males with isolated central fat accumulation and mixed forms of lipodystrophy had lower TT and SHBG levels.

In the female group, total abdominal fat showed a positive correlation with fT and a negative correlation with SHBG. SAT had a negative correlation with SHBG values and VAT presented a positive correlation with fT and a negative correlation with oestradiol. VAT/SAT ratio showed a negative correlation with oestradiol. No other significant association were observed as listed at table 4.

In males, a negative correlation between total abdominal fat and fT, androstenedione and oestradiol and a negative correlation with TT and with SHBG were observed. SAT showed a negative correlation with TT, fT, androstenedione and SHBG. VAT had a negative correlation with TT and

SHBG and a weaker negative correlation with oestradiol. VAT/SAT ratio presented with a very weak negative correlation with DHEA-S and with SHBG (Table 4).

Correlations with HOMA and hormones weren't significant, as showed in table 4.

Discussion:

Lipodystrophy is a well-known complication of cART and its prevalence increases over cART duration [4, 6]. Nevertheless, there are some reports of lipodystrophy in non-treated HIV patients, which supports the theory of a multifactorial aetiology for lipodystrophy [6]. The prevalence of this problem range between 10% and 85% [4] and it may be due to the different criteria and methodologies used to diagnose lipodystrophy.

New evidence indicates that lipodystrophy is more common in women than in men [21], although in our study lipodystrophy is more common in men (47.1% vs 29.2%).

In our sample, lipodystrophic men were older and no difference in age was observed in females. This was in accordance to the higher prevalence of lipodystrophy in older groups reported by others [6, 22]. Men with lipodystrophy presented lower SAT and higher VAT, similar to body composition changes observed in HIV-negative patients which are age related [6]. The changes seen in SAT and VAT may result in part from the natural aging process and as well from lipodystrophy HIV-associated, namely to cART. Furthermore, a cross-sectional study showed that, similarly to older non-HIV infected men (40-69 years), low TT levels are common in young/middle age HIV-infected men, mainly in those with lipodystrophy [11]. In our male patients, there were no differences in TT levels between those with or without lipodystrophy, suggesting that the age role in the presence of lipodystrophy may not be related to TT.

Longer HIV infection duration was associated with higher prevalence of lipodystrophy [6] but this wasn't observed in the present study. In our study, male patients with isolated lipoatrophy and mixed forms of lipodystrophy had longer HIV infection and duration of cART.

According to the literature, underweight became uncommon in HIV patients with successful therapy [18], and in our study, normal weight and overweight are more common in both genders. There was a greater prevalence of obesity among women in our population, which is in accordance to data of Eurostat for Portugal in non-HIV population female [23].

A previous work, involving both genders, reported a similar VAT, lower SAT and a higher VAT/SAT ratio between patients with and without lipodystrophy [18]. When analysed separately, the same happened in the female group but lipodystrophic males presented higher VAT. If VAT/SAT ratio in lipodystrophic women may be higher due to lower SAT, in men higher VAT may play a role too. When divided in four groups, isolated central fat had lower VAT/SAT ratio compared to isolated lipoatrophy because of higher SAT in despite of higher VAT. The lower VAT/SAT ratio in isolated lipohypertrophy probably occurs due to lower VAT and SAT. On the other hand, mixed forms showed the highest VAT/SAT ratio with highest VAT but an intermediate SAT.

The prevalence of insulin resistance in HIV adults under cART is estimated in about 37% [24]. A previous work described that nondiabetic insulin-resistant lipodystrophic HIV infected men have decreased values of TT, fT, oestradiol, SHBG and DHEA-S [5]. Wunder DM. et al, hypothesised that insulin resistance works as the link between lipoatrophy and hormonal changes, with longstanding insulin resistance decreasing the sensitivity of hypothalamic-pituitary-gonadal axis and consequently reducing testosterone secretion [12]. Lipohypertrophy seems to be linked with VAT and insulin resistance [6]. As expected, this study presents higher VAT values in isolated central fat accumulation and mixed forms, both associated with higher HOMA-IR in men. However, no

significant differences were seen in women. The absence of significant correlations between HOMA-IR and sexual hormones in this work is not in agreement with the positive correlation between TT and SHBG with insulin sensitivity described in another study that suggested an indirect hormonal mechanism in insulin sensitivity [5]. There was no significant correlation between sexual hormones and HOMA-IR. It raises the possibility that insulin-resistant may play a role in lipodystrophy aetiology but not directly related with sexual hormones.

Andersen et al. described a strong positive correlation between serum testosterone and serum oestradiol [5]. mRNA Aromatase (responsible for conversion from testosterone to oestradiol) are decreased in 50% in biopsies from abdominal SAT in men with lipodystrophy [5]. In our work, no significant differences were observed in oestradiol, TT or fT in men with or without lipodystrophy. Despite the decrease in mRNA aromatase in each cell, this depletion may be covert by the increased adipose tissue which can lead to a normal total aromatase production. This might explain the absence of significant differences in TT, fT and oestradiol when divided into groups according to lypodistrophy. Isolated abdominal accumulation and mixed forms which had higher total fat, SAT and VAT also had lower TT compared to the other two groups. This supports the idea that increased adipose tissue may neutralise the hormonal changes due to the lack of aromatase.

If the decrease in aromatase happened in women, it can influence the oestradiol levels in women with lipodystrophy although there are other causes are more likely to cause the decrease in oestradiol levels. In contrast to previous work that described an increased TT and fT in normal weight lipodystrophic women [13], no significant differences in TT or fT were observed in our sample. Others suggested that as TT and oestradiol have different measurement units (TT: nanograms/mL; oestradiol: pictograms/mL), molecule to molecule aromatization results in a relatively small alteration in TT comparatively to a big alteration in oestradiol [25], which can explain

the lower oestradiol not accompanied by an increased TT. Also, Andersen et al., observed a normal DHEA-S and a low oestradiol in men with lipodystrophy, hypothesizing that there was a failure in DHEA-S to oestradiol conversion thus explaining the lower oestradiol levels in this group [5].

Post-menopausal women have a decreased in oestradiol and increased abdominal adipose tissue which can be reversed with hormonal replacement therapy [5]. We also found a decreased oestradiol in isolated central fat accumulation and mixed forms which had an increased abdominal adipose tissue supporting the possible relation between oestradiol and body fat distribution and suggesting that hormonal replacement therapy may be beneficial to these two groups of lipodystrophic women. Also, women over 50 years only presented two phenotypes with higher total fat and VAT and this may be due to the normal changes in menopause or of small number of patients. Oestradiol deficiency related with aromatase mutation gene were associated with obesity [5]. This gives the idea that lipodistrophic women may have an aromatase deficiency (as proved for lipodistrophic men [5]) responsible for the observed lower oestradiol values. Furthermore, it was described that aromatase activity is higher, at least in SAT, in men compared to woman [25]. It's possible that, even with the aromatase deficiency, women may have a lower absolute aromatase value which could explain the higher obesity rate seen in female group.

Johnsen et al., described a negative relation between SHBG and CD4 count in female HIV subjects, which is in consonance to higher CD4 and lower SHBG seen in lipodystrophic men [26]. Another study, suggests that disease may be behind the variations seen in SHBG and may influence androgen levels [26]. This also could explain the controversy results in SHBG levels reported in literature, as SHBG seems to be related with disease control. Hadigan C. et al. suggests that highest SHBG levels are related with HIV infection and a variation in the other direction is due to the increase in truncal adiposity [13, 27]. VAT is associated with hyperinsulinemia which was linked with lower

SHBG liver production [28, 29]. A negative SHBG association with VAT and SAT is described non-HIV men and women [10, 30]. In conformity with these data, isolated central accumulation and mixed forms, characterized by higher VAT and HOMA-IR, had lower SHBG. Also, in both genders, SHBG had a negative correlation with total abdominal fat, and SAT. In men, SHBG showed a negative correlation with VAT and VAT/SAT ratio too. Also, a study including healthy post-menopausal women observed that variations in body mass were accompanied by variations in SHBG levels, with SHBG decreasing with weight gain and VAT [31]. We didn't find significant differences in correlation between VAT and SHBG in the female group. Obesity and namely visceral obesity is related with low levels of SHBG, but HIV-infection is associated with an increasing of SHBG levels, which could explain this [13, 27].

Larger prevalence studies demonstrated isolated central fat accumulation in HIV patients under cART in 30-62% [32] which is according to our results for the female group (44,4%) but only 16,9% men presented isolated central fat accumulation. Lipoatrophy prevalence ranges between 14% and 53% [22], which are according to our findings. Lipoatrophy seems to be more prevalent followed by mixed forms and lipohypertrophy [6] and the same was observed in this study for male group. Female group showed the opposite with lipohypertrophy being the most prevalent followed by mixed forms and the lipoatrophy, according to a Canadian study where female group lipohypertophy and mixed forms were the most common [21].

Polycystic ovary syndrome (PCOS) is associated with obesity (38-88%) and insulin resistance (50-90%) and usually presents with hyperandrogenaemia [29]. PCOS became clinically manifest with weigh gain in women with genetic predisposition [29]. Also, VAT is an independent predictor of metabolic syndrome and excessive VAT is associated with compensatory hyperinsulinemia and with a negative correlation with fT in non-infected individuals [10, 28]. Hyperinsulinemia interacts

synergistically with LH activating CYP17 leading to an increase in androgen production and releasing by ovary [29]. Hyperinsulinemia also increases LH amplitude pulse resulting in lower liver SHBG production [29]. Similar alterations were already described in lipodystrophic females [24]. The decrease in SHBG and the increase in androgen levels may lead to higher fT values justifying the fT positive correlation with VAT in women, although SHBG didn't show significant differences with VAT correlation. So, HIV infection may mimic PCOS, although no correlation were seen with HOMA-IR and sexual hormones. Nevertheless, the prevalence of PCOS is unknown in HIV population. Since obesity is associated with a higher frequency of this disease we can predict a possible higher prevalence of PCOS in HIV individuals.

We found a negative correlation between SAT and testosterone (TT and fT). An enlargement in adipose size in SAT were associated to lower testosterone levels in non-infected obese men [27]. Another study refers the possibility of VAT to predict inversely TT values [10, 11] according to our findings with a negative correlation between this two. They also suggests that VAT may be involved in suppression hypothalamic-pituitary axis resulting in low LH, also described in non-infected obese men, referring that weight loss and VAT reduction may decrease TT [11]. It was described an high LH in patients under cART with lipoatrophy but the increase in gonadotrophins did not result in higher fT values [12]. In fact, isolated lipoatrophy showed higher TT values in men and no significant differences in fT. As VAT seams to result in lower LH, it is no strange that isolated lipoatrophy and no lipodystrophy which had lower VAT will have higher LH and higher TT levels.

A study in healthy men with a two-fold increase in serum testosterone level, revealed a shift in triglyceride assimilation from VAT to SAT [5]. This may justify why groups with higher TT levels presented with lower VAT and the negative correlation between this two variables. The same doesn't apply for SAT which had a negative correlation with TT.

A placebo controlled 24-week trial with 10g testosterone, in HIV male population, was not associated with a significant reduction in VAT although total body fat and SAT had higher reduction when compared to placebo group [33]. This may happen due to a negative correlation observed between total body fat and SAT with fT but not with VAT (that occurs with TT). It's possible that VAT, by the suppression of the hypothalamic-pituitary axis have more influence in TT levels than de opposite.

It was described that men VAT had lower capacity of labelled oestradiol than men SAT [34]. Our search didn't show significant differences between the four types and oestradiol. Nevertheless, it seems to have a negative correlation among oestradiol and VAT and not SAT, suggesting that oestradiol may influence VAT by another pathway that may not be dependent of oestradiol receptors localized in VAT. In women, bidding capacity was equal in VAT and SAT although comparable with oestradiol bidding to VAT in men [34]. We observed higher oestradiol values for isolated lipoatrophy and no lipodystrophy and both had the lowest SAT and VAT values. In addition, a negative correlation was seen between oestradiol and VAT, stronger than that seen in men, as a negative correlation with VAT/SAT ratio. This reinforces the idea that oestradiol may influence the decrease of VAT more than SAT. Still, the effects of oestradiol in adipose tissue metabolism may depend on the type of receptor, which had different ratios in different adipose depots [5, 24, 35]. Polymorphisms associated with oestrogen receptors were related to sexual hormones levels and amount of body fat in non-infected men [36].

Studies in healthy men showed a negative correlation between VAT and DHEA [10]. Also, DHEA in elderly person leaded to a reduced abdominal fat [12]. The present study didn't find significant differences in correlation of VAT with DHEA-S, although DHEA-S seems to have a negative

correlation with VAT/SAT ratio. In healthy women, no significant negative correlation of DHEA-S with VAT were seen [10], as reported in HIV women in this study.

The present study had some limitations in part related to the observational design and the cross-sectional analyses, not allowing causal inference. This study sample were referenced to us due to lipodystrophy or metabolic disorders leading to a selection bias and so, our sample may overestimate this problems in HIV population. The body composition before HIV infection was not determined. Also, patients with hormonal disorders previous to HIV infection were not exclude. We didn't evaluate comorbidities which can influence hormonal output or body composition, such as PCOS. An important determinant of body composition, physical activity, were not evaluated. In female group, post-menopausal women were estimated by age which is not the best parameter especially in HIV women.

This study has some strong points. It was performed in a highly experience unit in the assessment of metabolic and body fat abnormalities in HIV-infected patients, with all clinical assessments being executed by the same practitioner. An objective definition of lipodystrophy was used (fat mass ratio by DEXA) and visceral fat by CT. Moreover, the sample size was relatively large compared to other studies in the same area.

Conclusion:

In summary, body distribution in HIV patients under cART seems to be, at least in part, related with sex hormones and different associations were observed in both genders. Lower oestradiol values in women and lower SHBG values in men are related with presence of lipodystrophy especially with central fat accumulation. TT seems to be important to lipodystrophy phenotype in

men, being lower in patients with central fat accumulation. Insulin resistance is important in pathophysiology of lipodystrophy although it may not be directly related with sexual hormones.

List of abbreviations:

BMI- body mass index; cART- antiretroviral therapy; CDC- Centers for Disease Control and Prevention; DEXA- dual-energy X-ray absorptiometry; DHEA-S- dehydroepiandrostenedione-sulfate; FMR- fat mass ratio; fT- free testosterone; HIV- human immunodeficiency virus; HOMA-IR - homeostasis model assessment of insulin resistance; ICFA- isolated central fat accumulation; IL- isolated lipoatrophy; IR- interquartile range; LPD- lipodystrophy; MF- mixed forms; r- correlation coefficient; SAT- subcutaneous adipose tissue; SD- standard deviation; SHBG- sex hormone-binding globulin; TT- total testosterone; VAT- visceral adipose tissue;

Ethics approval and consent to participate:

All patients provided written informed consent and the study protocol was approved by the São João Hospital's Ethics Committee for Health.

Consent for publication:

Not applicable.

Availability of data and materials:

The data that support the findings of this study are available on request from the corresponding author [ARG]. The data are not publicly available because they contain information that could compromise research participant privacy/consent.

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Table 1. Sample characteristics by gender according to presence and absence of clinical lipodystrophy determined by FMR.

	Men			Women		
	Without LPD	**With LPD**	**p**	**Without LPD**	**With LPD**	**p**
N (frequency, %)	72 (52.9)	64 (47.1)		51 (70.8)	21 (29.2)	
Age [years, mean (SD)]	45.10 (12.02)	49.09 (9.88)	**0.037**	44.57 (12.87)	50.86 (10.82)	0.053
HIV infection duration [years, mean (SD)]	7.28 (4.03)	8.28 (3.44)	0.123	8.0 (4.0)	9.95 (3.81)	0.061
HIV stage [n (%)]						
A	26 (36.1)	24 (37.5)		25 (49)	11 (52.4)	
B	20 (27.8)	25 (39.1)		8 (15.7)	2 (9.5)	
C	26 (36.1)	15 (23.4)	0.209	18 (35.3)	8 (38.1)	0.877
CD4 count [cells/mm3, mean (SD)]	485.36 (281.18)	617.23 (325.87)	**0.012**	494.82 (284.84)	715.05 (400.43)	**0.029**
Viral load (<50 copies/mL) [n (%)]	61 (100)	58 (100)		41 (100)	19 (100)	
cART duration [years, mean (SD)]	5.94 (4.06)	7.77 (3.42)	**0.006**	5.57 (3.66)	9.19 (3.16)	**<0.001**
Smoking history [n (%)]						
Current	18 (25)	21 (32.8)		30 (58.8)	15 (71.4)	
Former	39(54.2)	27 (42.2)		19 (37.3)	3 (14.3)	
Never	15 (20.8)	16 (25)	0.371	2 (3.9)	3 (14.3)	0.063
Alcohol consumption [n (%)]	33 (45.8)	31 (48.4)	0.761	8 (15.7)	2 (9.5)	0.71
BMI [(kg/m2), mean (SD)]	24.53(3.92)	25.16 (3.55)	0.329	26.30 (5.72)	26.12 (5.25)	0.903
Underweight [n (%)]	4 (5.6)	2 (3.1)		3 (5.9)	2 (9.5)	
Normal weight [n (%)]	40 (55.6)	32 (50)		18 (35.3)	8 (38.1)	
Overweight [n (%)]	21(29.2)	24 (37.5)		20 (39.2)	5 (23.8)	
Obesity [n (%)]	7 (9.7)	6 (9.4)	0.743	10 (19.6)	6 (28.6)	0.590
Waist circumference [cm, mean (SD)]	90.44 (10.98)	92.84 (10.07)	0.188	90.86 (13.68)	93.29 (12.12)	0.483
Hip circumference [cm, mean (SD)]	94.28 (7.05)	92.39 (6.34)	0.105	99.08 (11.04)	93.38 (8.19)	**0.036**
Thigh circumference [cm, mean (SD)]	47.85 (4.27)	46.85 (3.90)	0.159	49.71 (6.73)	47.12 (7.18)	0.149
Arm circumference [cm, mean (SD)]	(n=57) 26.68 (2.59)	(n=55) 27.42 (2.35)	0.115	(n=44) 26.73 (3.86)	(n=21) 26.74 (3.35)	0.991
Body fat mass by quantitative CT [frequency (%)]	67 (52.34)	61(47.66)		42 (66.67)	21 (33.33)	
Total fat [cm2, mean (SD)]	243.09 (163.69)	251.13 (95.06)	0.732	356.93 (168.50)	368.42 (157.10)	0.795
VAT [cm2, mean (SD)]	121.26 (96.77)	166.70 (66.26)	**0.002**	98.73 (62.83)	148.29 (70.86)	**0.006**
SAT [cm2, median (IR)]	105.37 (94.67)	68.60 (60.61)	**0.012**	235.64 (166.75)	211.67 (129.39)	0.262
VAT/SAT ratio [cm2, median (IR)]	1.00 (1.22)	2.41(1.96)	**<0.001**	0.36 (0.34)	0.67 (0.37)	**0.001**
HOMA IR [median, (IR)]	1.68 (2.85)	2.63 (2.78)	**0.045**	1.54(1.37)	3.07 (4.13)	**0.016**
TT [ng/dL, median (IR)]	6.39 (4.22)	5.37 (2.58)	0.134	0.4 (0.30)	0.35 (0.5)	0.232
fT [pg/mL, median (IR)]	14.24 (10.26)	14.88 (4.65)	0.306	0.54 (0.72)	0.5 (0.92)	0.692
DHEA-S [ug/dL, median (IR)]	150.0 (170.15)	171.9 (170.7)	0.240	146.5 (116.4)	123.95 (102.3)	0.513
Androstenedione [ng/mL, median (IR)]	2.26 (1.67)	2.45 (1.27)	0.075	1.63 (1.05)	1.25 (0.83)	0.078
SHBG [nmol/L, median (IR)]	59.3 (52.8)	42.9 (25.0)	**0.002**	85.90 (54.80)	65.55 (56.5)	0.442
Oestradiol [pg/mL, median (IR)]	29.1 (14.9)	26.0 (13.5)	0.311	57 (103.0)	24.1 (60.8)	**0.039**

(LPD- lipodystrophy; SD- standard deviation; cART- antiretroviral therapy; BMI- body mass index; VAT- visceral adipose tissue; SAT- subcutaneous adipose tissue; IR- interquartile range; HOMA-IR- homeostasis model assessment of insulin resistance; TT- total testosterone; fT- free testosterone; DHEA-S- dehydroepiandrostenedione-sulfate; SHBG- sex hormone-binding globulin)

Table 2. Body composition and sex hormones according the four different groups in body composition in women

	No lipodystrophy	Isolated central fat accumulation	Isolated lipoatrophy	Mixed forms of lipodystrophy	p
N (frequency, %)	4 (5.6)	32 (44.4)	10 (13.9)	26 (36.1)	
HIV infection duration [years, median (IR)]	7.0 (3.8)	7.5 (5.8)	10 (6.5)	10.0 (5.3)	0.197
cART duration [years, median (IR)]	5.5 (6.8)	4.0 (5.0)	9.0 (5.3)	9.0 (4.0)	**0.002**
Waist circumference [cm, median (IR)]	76.0 (5.0)	97.5 (14.8)	72.5 (6.3)	91.5 (11.5)	**<0.001**
Hip circumference [cm, median (IR)]	93.5 (5.5)	104.0 (8.0)	85.0 (3.5)	91.5 (5.3)	**<0.001**
Thigh circumference [cm, median (IR)]	47.5 (9.3)	52.5 (8.5)	42.0 (2.3)	46.5 (5.0)	**<0.001**
Arm circumference [cm, median (IR)]	23.0	29.0 (4.3)	22.5 (3.3)	27.0 (4.0)	**<0.001**
Total fat [cm2, median (IR)]	167.58 (160.35)	396.08 (200.14)	148.38 (116.15)	301.62 (159.17)	**<0.001**
VAT [cm2, median (IR)]	43.47 (39.49)	105.82 (81.85)	51.37 (24.57)	136.8 (81.92)	**<0.001**
SAT [cm2, median (IR)]	123.79 (128.37)	321.14 (170.59)	93.33 (95.12)	205.3 (72.34)	**<0.001**
VAT/SAT ratio [cm2, median (IR)]	0.31 (0.16)	0.34 (0.21)	0.56 (0,46)	0.63 (0,39)	**0.004**
HOMA IR [median (IR)]	1.37 (0.91)	1.62 (2.20)	0.96 (1.37)	2.52 (4.03)	0.065
TT [ng/dL, median (IR)]	0.38 (0.18)	0.43 (0.35)	0.35 (0.23)	0.36 (0.59)	0.793
fT [pg/mL, median (IR)]	0.48 (0.80)	0.55 (0.78)	0.33 (0.75)	0.52 (0.66)	0.571
DHEA-S [ug/dL, median (IR)]	208.2 (106.07)	148.65 (119.82)	146.0 (77.63)	108.0 (113.75)	0.155
Androstenedione [ng/mL, median (IR)]	1.64 (0.62)	1.65 (1.13)	1.58 (1.24)	1.22 (0.95)	0.378
SHBG [nmol/L, median (IR)]	153.3 ()	81.0 (47.0)	95.50 (34.13)	75.95 (56.83)	0.174
Estradiol [pg/mL, median (IR)]	94.15 (107.8)	55.95 (105.9)	110.30 (134.0)	24.2 (44.1)	**0.012**
<50 years	94.15 (107.8)	76.2 (79.8)	110.3 (134.0)	47.05 (62.5)	**<0.001**
>50 years	--	9.9 (0)	--	11.6 (13.2)	0.999

(IR- interquartile range; cART- antiretroviral therapy; VAT- visceral adipose tissue; SAT- subcutaneous adipose tissue; HOMA-IR - homeostasis model assessment of insulin resistance; TT- total testosterone; fT- free testosterone; DHEA-S- dehydroepiandrostenedione-sulfate; SHBG- sex hormone binding globulin)

Table 3. Body composition and sex hormones according the four different groups in body composition in men

	No lipodystrophy	Isolated central fat accumulation	Isolated lipoatrophy	Mixed forms of lipodystrophy	p
N (frequency, %)	17 (12.5)	23 (16.9)	62 (45.6)	34 (25)	
HIV infection duration [years, median, (IR)	8.00 (3.00)	5.00 (3.0)	9.00 (5.0)	7.50 (4.3)	**<0.001**
cART duration [years, median (IR)]	4.00 (4.5)	4.00 (4.0)	8.00 (5.0)	7.50 (4.3)	**<0.001**
Waist circumference [cm, median (IR)]	85.00 (6.0)	100.00 (11.0)	85.00 (9.0)	99.00 (8.5)	**<0.001**
Hip circumference [cm, median (IR)]	93.00 (6.5)	100.00 (9.0)	89.00 (7.0)	96.00 (6.3)	**<0.001**
Thigh circumference [cm, median (IR)]	48.00 (5.0)	52.00 (5.0)	46.00 (4.5)	49.00 (4.0)	**<0.001**
Arm circumference [cm, median (IR)]	25.00 (5.0)	28.00 (2.5)	26.50 (3.6)	28.25 (2.6)	**<0.001**
Total fat [cm2, median (IR)]	188.42 (95.08)	395.68 (140.58)	146.4900 (111.08)	304.80 (150.39)	**<0.001**
VAT [cm2, median (IR)]	70.32 (88.54)	180.34 (164.6)	97.57 (86.81)	203.43 (86.82)	**<0.001**
SAT [cm2, median (IR)]	100.27 (55.38)	171.45 (128.59)	57.43 (53.21)	93.45 (69.38)	**<0.001**
VAT/SAT ratio [cm2, median (IR)]	0.65 (0.52)	0.94 (1.13)	1.72 (2.45)	2.08 (1.50)	**<0.001**
HOMA IR [median (IR)]	1.23 (1.59)	3.45 (5.04)	1.85 (2.79)	3.05 (3.77)	**0.005**
TT [ng/dL, median (IR)]	6.40 (4.44)	5.37 (3.56)	6.96 (3.95)	4.79 (2.24)	**0.003**
fT [pg/mL, median (IR)]	17.13 (11.60)	14.34 (9.87)	14.86 (6.82)	14.40	0.190
DHEA-S [ug/dL, median (IR)]	201.7 (160.7)	140.2 (169.2)	155.05 (143.2)	155.65 (190.30)	0.199
Androstenedione [ng/mL, median (IR)]	2.41 (1.57)	2.18 (1.31)	2.57 (1.71)	2.18 (1.71)	0.162
SHBG [nmol/L, median (IR)]	54.45 (67.98)	35.85 (21.48)	60.70 (58.05)	40.50 (21.10)	**<0.001**
Estradiol [pg/mL, median (IR)]	24.65 (15.7)	26.0 (18.0)	31.0 (11.8)	24.5 (9.5)	0.052

(IR- interquartile range; cART- antiretroviral therapy; VAT- visceral adipose tissue; SAT- subcutaneous adipose tissue; HOMA-IR- homeostasis model assessment of insulin resistance; TT- total testosterone; fT- free testosterone; DHEA-S- dehydroepiandrostenedione-sulfate; SHBG- sex hormone binding globulin)

Table 4 - Spearman's correlation between androgens, body composition and HOMA-IR in women and men

		TT (n_1=59; n_2=115)		Tf (n_1=59, n_2=117)		DHEA-S (n_1=60, n_2=122)		Androstenedione (n_1=61, n_2=121)		SHBG (n_1=48, n_2=100)		Estradiol (n_1=61, n_2=120)	
		r	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Total abdominal fat	**Women**	-0.03	0.83	0.27	**0.036**	-0.21	0.11	0.01	0.92	-0.37	**0.009**	-0.24	0.07
	Men	-0.34	**<0.001**	-0.21	**0.022**	-0.13	0.15	-0.23	**0.012**	-0.41	**<0.001**	-0.22	**0.02**
SAT	**Women**	-0.10	0.45	0.23	0.082	-0.22	0.09	-0.004	0.99	-0.35	**0.016**	-0.08	0.53
	Men	-0.23	**0.012**	-0.22	**0.015**	-0.01	0.89	-0.21	**0.024**	-0.22	**0.028**	-0.16	0.08
VAT	**Women**	0.01	0.93	0.26	**0.045**	-0.14	0.30	0.02	0.91	-0.27	0.066	-0.45	**<0.001**
	Men	-0.38	**<0.001**	-0.18	0.06	-0.68	0.07	-0.16	0.086	-0.42	**<0.001**	-0.19	**0.04**
VAT/SAT	**Women**	0.07	0.59	0.07	0.60	0.03	0.83	-0.01	0.96	0.01	0.96	-0.38	**0.002**
	Men	-0.13	0.17	0.066	0.48	-0.18	**0.05**	0.02	0.81	-0.20	**0.05**	0.01	0.95
HOMA IR	**Women**	-0.13	0.29	-0.003	0.98	-0.03	0.82	-0.09	0.46	-0.03	0.83	-0.,17	0.17
	Men	-0.18	0.051	-0.13	0.16	-0.09	0.33	0.10	0.27	-0.13	0.17	0.11	0.17

(n_1-women frequency; n_2 – men frequency, TT- total testosterone; fT- free testosterone; DHEA-S- dehydroepiandrostenedione-sulfate; SHBG- sex hormone binding globulin; r- correlation coefficient; VAT- visceral adipose tissue; SAT- subcutaneous adipose tissue.)

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Anexos:

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Keywords: Three to ten keywords representing the main content of the article.

Background: The Background section should explain the background to the study, its aims, a summary of the existing literature and why this study was necessary or its contribution to the field.

Methods: The methods section should include:

- the aim, design and setting of the study
- the characteristics of participants or description of materials
- a clear description of all processes, interventions and comparisons. Generic drug names should generally be used. When proprietary brands are used in research, include the brand names in parentheses
- the type of statistical analysis used, including a power calculation if appropriate

Results: This should include the findings of the study including, if appropriate, results of statistical analysis which must be included either in the text or as tables and figures.

Discussion: This section should discuss the implications of the findings in context of existing research and highlight limitations of the study.

Conclusions: This should state clearly the main conclusions and provide an explanation of the importance and relevance of the study reported.

List of abbreviations: If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations should be provided.

Declarations: All manuscripts must contain the following sections under the heading 'Declarations': ethics approval and consent to participate, consent for publication, availability of data and material, competing interests, funding, authors' contributions, acknowledgements, authors' information (optional), please see below for details on the information to be included in these sections. If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

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transplant. RH performed the histological examination of the kidney, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript."

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Article within a journal: Smith JJ. The world of science. Am J Sci. 1999;36:234-5.

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Article within a journal by DOI: Slifka MK, Whitton JL. Clinical implications of dysregulated cytokine production. Dig J Mol Med. 2000; doi:10.1007/s801090000086.

Article within a journal supplement: Frumin AM, Nussbaum J, Esposito M. Functional asplenia: demonstration of splenic activity by bone marrow scan. Blood 1979;59 Suppl 1:26-32.

Book chapter, or an article within a book: Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. In: Bourne GH, Danielli JF, Jeon KW, editors. International review of cytology. London: Academic; 1980. p. 251-306. OnlineFirst chapter in a series (without a volume designation but with a DOI) Saito Y, Hyuga H. Rate equation approaches to amplification of enantiomeric excess and chiral symmetry breaking. Top Curr Chem. 2007. doi:10.1007/128_2006_108.

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Dataset with persistent identifier: Zheng L-Y, Guo X-S, He B, Sun L-J, Peng Y, Dong S-S, et al. Genome data from sweet and grain sorghum (Sorghum bicolor). GigaScience Database. 2011. http://dx.doi.org/10.5524/100012.

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329-16

Unidade de Investigação

Tomei conhecimento. Nada a opor.

04 de Janeiro de 2017

A Coordenadora da Unidade de Investigação

(Prof.ª Doutora Ana Azevedo)

AUTORIZADO

CONSELHO DE ADMINISTRAÇÃO (●) REUNIÃO DE 12 JAN 2017

Presidente do Conselho de Administração

Diretor Clínico | Enfermeira Diretora | Vogal Executivo | Vogal Executivo

Exmo. Senhor

Presidente do Conselho de Administração do

Centro Hospitalar de S. João – EPE

DIRECÇÃO CLINICA

10 JAN 2017

Aprovado. Ao CA.

(Prof.ª Doutora Ana Azevedo)

Assunto: Pedido de autorização para realização de estudo/projecto de investigação

Nome do Investigador Principal: Maria Leonor Pires da Silva

Título do projecto de investigação: Gender different association of androgens to body fat mass distribution in HIV infected patients

Pretendendo realizar no(s) Serviço(s) de Endocrinologia do Centro Hospitalar de S. João – EPE o estudo/projecto de investigação em epígrafe, solicito a V. Exa., na qualidade de Investigador/Promotor, autorização para a sua efectivação.

Para o efeito, anexa toda a documentação referida no dossier da Comissão de Ética do Centro Hospitalar de S. João respeitante a estudos/projectos de investigação, à qual endereçou pedido de apreciação e parecer.

Com os melhores cumprimentos.

Porto, 7 / Novembro / 2016

O INVESTIGADOR/PROMOTOR

Maria Leonor Pires da Silva

Comissão de Ética para a Saúde do HSJ/FMUP
Parecer

Projeto de investigação: "Gender difference association of androgens to body fat mass distribuition in HIV infected patients".

Promotores:
- Não aplicável.

- Pertinência do estudo

- Trata-se de um estudo observacional, retrospectivo, a realizar no âmbito da tese de Mestrado Integrado em Medicina na Faculdade de Medicina da Universidade do Porto (FMUP), que tem como objectivo principal, avaliar a relação entre lipodistrofia definida pelo *Fat Mass Ratio*, as 4 categorias de distribuição de gordura corporal e as hormonas sexuais (testosterona total, testosterona livre, DHEA-D, androstenodiona, SHBG e estradiol).
- Tem ainda como outros objectivos estudar um grupo de homens e de mulheres com infecção VIH, com de sem lipodistrofia, sob terapêutica antiretrovírica combinada (TARc): comparando a redistribuição da gordura corporal; comparando as alterações das hormonas sexuais; avaliando a insulinorresistência; outros aspectos pertinentes e relacionados com os objectivos do estudo.
- Este estudo surge na sequência de um trabalho previamente autorizado pela CES "Estudo do Síndrome da lipodistrofia e das repercussões endócrino-metabólicas e cardiovasculares na infecção VIH" e que desta vez fará uma abordagem transversal dos dados recolhidos no trabalho mencionado, incluindo 621 adultos com infecção VIH serologicamente documentada a realizar TARc (n=430 homens; n=177 mulheres).
- Todos os dados a colher de forma anónima (sócio-demográficos, antropométricos, clínicos, virológicos, analíticos e terapêuticos) são pertinentes e adequados aos objectivos do estudo.
- O estudo é pertinente, importante e está muito bem fundamentado.
- O protocolo de estudo, os critérios de inclusão e de exclusão estão suficientemente detalhados e não levantam quaisquer questões do foro ético.
- A Investigadora Principal, Maria Leonor Pires da Silva, estudante do 6º ano do curso de Medicina da FMUP, tendo como elo de ligação (e orientadora da Tese) a Médica especialista de Endocrinologia, a Professora Paula Freitas (especialista do Serviço de Endocrinologia do Hospital de S. João EPE), dispõe das competências técnica e científica para a realização do estudo.
- O estudo será realizado no Serviço de Endocrinologia do Hospital de S. João, EPE e dispõe da autorização para a sua realização pelo seu Director, Professor Davide Carvalho. O serviço proponente dispõe das condições necessárias para a realização do estudo.

– Beneficio/Risco

- Dada a natureza retrospectiva do estudo, não haverá riscos, incómodos ou benefícios para os participantes.

– **Respeito pela liberdade e autonomia do sujeito do ensaio**

- Dada a natureza retrospectiva do estudo, não há necessidade de proceder à obtenção do consentimento informado.

– **Confidencialidade dos dados**

- A confidencialidade e a privacidade dos dados são garantidas.

– **Indemnização por danos**

Não aplicável.

– **Continuação do tratamento**

Não aplicável.

- **Propriedade dos dados**

Não aplicável.

Conclusão

Em face da análise do protocolo de "Gender difference association of androgens to body fat mass distribuition in HIV infected patients", proponho a sua aprovação pela CES do HSJ/FMUP.

Porto, 18 de novembro de 2016

O Relator
Prof. Manuel Vaz Silva

7. SEGURO

a. *Este estudo/projecto de investigação prevê intervenção clínica que implique a existência de um seguro para os participantes?*

SIM ☐ (Se sim, junte, por favor, cópia da Apólice de Seguro respectiva)
NÃO ☒
NÃO APLICÁVEL ☐

8. TERMO DE RESPONSABILIDADE

Eu, Maria Leonor Pires da Silva,
abaixo-assinado, na qualidade de Investigador Principal, declaro por minha honra que as informações prestadas neste questionário são verdadeiras. Mais declaro que, durante o estudo, serão respeitadas as recomendações constantes da Declaração de Helsínquia (com as emendas de Tóquio 1975, Veneza 1983, Hong-Kong 1989, Somerset West 1996 e Edimburgo 2000) e da Organização Mundial da Saúde, no que se refere à experimentação que envolve seres humanos. Aceito, também, a recomendação da CES de que o recrutamento para este estudo se fará junto de doentes que não tenham participado em outro estudo no decurso do actual internamento ou da mesma consulta.

Porto, 7 / Novembro / 2016

Maria Leonor Pires da Silva
O Investigador Principal

PARECER DA COMISSÃO DE ÉTICA PARA A SAÚDE DO CENTRO HOSPITALAR DE S. JOÃO

emitido na reunião plenária da CES de 18 / Novembro / 2016

A Comissão de Ética para a Saúde APROVA por unanimidade o parecer do Relator, pelo que nada tem a opor à realização deste projecto de investigação.