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SHORT COMMUNICATION

Androgen and gonadotropin patterns differ in HIV-1-infected men who develop lipoatrophy during antiretroviral therapy: a case–control study

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Objectives

We compared androgen and gonadotropin values in HIV-infected men who did and did not develop lipoatrophy on combination antiretroviral therapy (cART).

Methods

From a population of 136 treatment-naïve male Caucasians under successful zidovudine/lamivudine-based cART, the 10 patients developing lipoatrophy (cases) were compared with 87 randomly chosen controls. Plasma levels of free testosterone (fT), dehydroepiandrosterone (DHEA), follicle-stimulating hormone and luteinizing hormone (LH) were measured at baseline and after 2 years of cART.

Results

At baseline, 60% of the cases and 71% of the controls showed abnormally low fT values. LH levels were normal or low in 67 and 94% of the patients, respectively, indicating a disturbance of the hypothalamic–pituitary–gonadal axis. fT levels did not significantly change after 2 years of cART. Cases showed a significant increase in LH levels, while controls showed a significant increase in DHEA levels. In a multivariate logistic regression model, lipoatrophy was associated with higher baseline DHEA levels ($P = 0.04$), an increase in LH levels during cART ($P = 0.001$), a lower body mass index and greater age.

Conclusions

Hypogonadism is present in the majority of HIV-infected patients. The development of cART-related lipoatrophy is associated with an increase in LH and a lack of increase in DHEA levels.

Keywords: antiretroviral therapy, dehydroepiandrosterone, follicle-stimulating hormone, free testosterone, HIV, lipoatrophy, lipodystrophy, luteinizing hormone

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Introduction

Hypogonadism, mainly of the hypogonadotropic form, is frequent in HIV-positive males [1–3]. The impact of successful combination antiretroviral therapy (cART) on the hypothalamic–pituitary–gonadal (HPG) axis is still poorly defined. Also, little is known about the influence of sex hormone levels on the development of lipodystrophy.

*See Appendix.

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The lipodystrophy syndrome represents a well-known adverse effect of cART consisting of fat redistribution abnormalities, insulin resistance and dyslipidaemia [4,5]. Lipodystrophy includes central lipohypertrophy and peripheral lipoatrophy. The latter has been associated with the toxic effects of nucleoside reverse transcriptase inhibitors (NRTIs) on mitochondria [6–8] and cART-related dysregulation of the expression of cytokines and cytokine receptors, and there are some reports of genetic predispositions, based on single nucleotide polymorphisms, to cART-related lipoatrophy [6,9–12].

We showed in a previous study that there is no association between changes in leptin levels and the development of lipoatrophy [13]. In this study, we evaluated the association between sex hormone levels and the development of lipoatrophy in HIV-infected men treated with cART.

Materials and methods

The Swiss HIV Cohort Study (SHCS; www.shcs.ch) prospectively follows HIV-infected adults. Every 6 months, clinical data are collected and blood plasma samples are drawn, analysed and stored at $-80\ ^{\circ}C$ for future research.

Study design

We performed a nested case–control study in HIV-infected, antiretroviral therapy-naïve Caucasian men who were successfully treated with zidovudine (ZDV)- and lamivudine (3TC)-based cART for a minimum of 2 years. Patients with acute opportunistic infections, uncontrolled AIDS-defining illnesses, diabetes mellitus, androgen treatment or a change in cART during the study period were excluded. Cases developing lipoatrophy and negative controls were identified and serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), free testosterone (fT) and dehydroepiandrosterone (DHEA) were measured in the frozen plasma samples drawn before (baseline) and nearest to 2 years after the start of cART. Lipoatrophy was diagnosed when the patient and the treating physician agreed that fat loss had occurred in one or more of the following locations: face (buccal, periorbital and temporal), arms, legs or buttocks.

Measurement of hormones in the plasma

Hormone measurements after frozen storage have been validated [14]. All measurements were performed in duplicate according to the manufacturers' protocols. FSH and LH were quantified by commercial microplate double-antibody enzyme-linked immunosorbent assay (ELISA) methods (Easia, Medgenix, Fleurus, Belgium). DHEA was determined with a competitive, single-antibody microplate ELISA (Morwell Diagnostics, Zurich, Switzerland). fT was measured by radio-immunoassay (RIA) (Morwell Diagnostics) [3]. Age-adjusted normal ranges were used for the categorization of values.

Statistical analysis

Nonparametric tests were used for numerical data and χ^2 statistics for categorical data. Possible associations of individual parameters with the development of lipoatrophy were evaluated in a backward stepwise logistic regression model with a significance level of 0.2 for the removal of variables. Variables included baseline LH, FSH and DHEA values, their absolute changes, the proportion of fT levels below the normal range, age, body mass index and baseline CD4 cell and HIV RNA counts. A two-sided *P*-value of 0.05 or less was accepted as significant.

Results

Patient characteristics

Among the 136 patients fulfilling the inclusion criteria, we identified all patients with lipoatrophy ($n = 10$) and randomly selected 87 controls. Cases and controls did not differ by HIV transmission mode, Centers for Disease Control and Prevention stage (20 and 24% at stage B and 10 and 5% at stage C, respectively) or median body mass index either at baseline [22.7 (interquartile range {IQR} 21.8–24.9) and 23.6 (IQR 20.4–26.1), respectively] or after 2 years of cART. Their median ages were 44 years (IQR 41–45 years) and 40 years (35–45 years), respectively. There was a trend towards higher baseline CD4 cell counts in cases, with a median of 380 (IQR 173–558) cells/μL in cases compared with 200 (IQR 94–322) cells/μL in controls ($P = 0.09$). The third ARV drug in addition to ZDV and 3TC was a protease inhibitor in the majority of the patients, but was a nonnucleoside reverse transcriptase inhibitor in four (40%) of the cases and in 32 (37%) of the controls.

Hormone plasma concentrations at baseline and after 2 years of cART

Hormone levels before and after 2 years of cART are shown in Table 1. Before the start of cART, there were no statistically significant differences between cases and controls, neither for absolute hormone levels nor for the proportions of levels outside the normal range (data not shown).

Table 1 Hormone levels before and after 2 years of combination antiretroviral therapy (cART)

	Cases ($n = 10$)	Controls ($n = 87$)	*P*-value
FSH (IU/L)			
Before cART	2.9 (1.6–5.1)	3.1 (1.5–5.2)	0.9
After 2 years of cART	3.6 (3.2–5.8)	2.6 (1.8–4.7)	0.1
P-value	0.06	0.2	
LH (IU/L)			
Before cART	2.8 (1.9–3.3)	3.1 (1.7–5.0)	0.8
After 2 years of cART	7.2 (3.7–8.6)	3.0 (1.9–5.6)	0.004
P-value	0.005	0.9	
DHEA (ng/mL)			
Before cART	7.2 (4.1–9.1)	6.2 (4.3–9.4)	0.8
After 2 years of cART	7.1 (5.0–10.2)	8.2 (5.0–13.2)	0.5
P-value	0.8	<0.001	
fT (pmol/L)			
Before cART	24.9 (15.3–32.1)	22.9 (17.6–28.8)	NA
After 2 years of cART	24.7 (16.1–35.8)	23.7 (18.0–31.7)	NA
P-value	0.8	0.7	

Values are medians (interquartile ranges).
NA, not applicable; absolute values of free testosterone cannot be compared between cases and controls because of age-dependent normal values.
DHEA, dehydroepiandrosterone; FSH, follicle-stimulating hormone; fT, free testosterone; LH, luteinizing hormone.

fT and gonadotropins

At baseline, six of the 10 cases (60%) and 63 of the 87 controls (72%) had fT levels below the age-adjusted normal range, indicating hypogonadism. In 67% (cases) and 92% (controls) of these patients with low fT, the corresponding LH values were normal or low, indicating normo- or hypogonadotropic hypogonadism. After 2 years of cART, the rate of hypogonadism had remained stable, at 50% in patients with lipoatrophy ($P = 0.8$) and 64% in patients without lipoatrophy ($P = 0.7$). By that time, FSH ($P = 0.06$) and LH ($P = 0.005$) levels had increased in patients who developed lipoatrophy, but had remained unchanged or tended to be lower in controls (Table 1). The rate of below-normal LH, for example, was reduced from 40 to 0% in cases, while it remained stable in controls (33 *vs*. 29%). Consequently, hypogonadism was less frequently hypo- or normogonadotropic in lipoatrophic patients after 2 years of cART (20%), as compared with controls (90%).

DHEA

Baseline DHEA levels were normal in 80% of cases and 78% of controls. Under treatment, only the controls showed a significant increase in above-normal DHEA values from 14 to 30% ($P = 0.001$; Table 1).

Association of hormone levels and development of lipoatrophy

In a logistic regression model, the development of lipoatrophy was associated with greater age, a lower baseline body mass index, a higher baseline DHEA level and a more pronounced increase in LH levels during cART (Table 2). These results did not change when leptin levels were introduced as a covariate (data not shown) [13].

Table 2 Factors associated with the development of lipoatrophy during 2 years of combination antiretroviral therapy (cART) according to the results of the backward stepwise logistic regression model

Parameter	Adjusted OR (95% CI)	*P*-value
Age (per quartile increase)	3.6 (1.4–9.3)	0.01
CD4 count (per increase of 50 cells/μL)	1.2 (0.95–1.4)	0.12
Body mass index (per unit increase)	0.71 (0.5–0.98)	0.04
Increase of LH level during cART (per IU/L increase)	1.9 (1.3–2.9)	0.001
Baseline DHEA level (per ng/mL increase)	1.2 (1.02–1.3)	0.04

CI, confidence interval; DHEA, dehydroepiandrosterone; LH, luteinizing hormone; OR, odds ratio.

Discussion

Our study has provided two important findings. First, hypogonadism occurred in a majority of HIV-positive men and did not resolve under cART [3]. Secondly, the development of lipoatrophy was associated with a distinct endocrinological pattern that was not seen in patients without lipoatrophy: a significant increase in LH and a lack of increase in DHEA levels.

Our study has strengths and limitations. One of the strengths is the choice of participants in the study, which produced a unique and relatively uniform study population: (i) all patients were ARV-naïve; (ii) they started and continued for at least 2 years, without changes, on the NRTI backbone zidovudine/lamivudine, which guaranteed a stable pharmacological influence; and (iii) they continued to have undetectable HIV RNA during the study period, which is a surrogate for good adherence to therapy. Furthermore, although the chosen NRTI backbone is associated with development of lipoatrophy, the association is much less strong than that for stavudine-based ARV regimens [15]. Therefore, we hypothesized that our cases were likely to be especially prone to develop this disorder based on their genetic and metabolic background. Limitations of our study include, first, the use of frozen plasma samples that were not uniformly taken in a morning fasting state. This precluded additional analyses to further determine insulin resistance or include measurement of parameters with marked diurnal variations. Secondly, we have no objective measurements of limb fat loss such as dual-energy X-ray absorptiometry but rely on the subjective clinical definition as defined in our cohort. Therefore, we probably missed patients with subtle lipoatrophy

and were not able to quantify fat loss reliably. Thirdly, because of the strict study entry criteria, the number of cases was rather small, and so our findings should be confirmed in a larger study.

At baseline, hypogonadism was mostly associated with low or normal gonadotropin levels, indicating a disturbance of the HPG axis. In previous studies, androgen deficiency in HIV-positive men has been associated with low CD4 cell counts, a more advanced stage of disease and weight loss [16–18]. In our study, cART was associated with an increase in gonadotropin levels in patients who developed lipoatrophy ($P = 0.005$ for LH; $P = 0.06$ for FSH), but not in negative controls ($P = 0.2$ for LH; $P = 0.9$ for FSH). This increase in gonadotropin levels, however, did not result in increased fT. Similarly, most [18] but not all [19] previous reports described no effect of cART on hypogonadism. The limitations of our study include the use of stored plasma samples, because they were not all taken in a morning fasting state. Diurnal variations, with lower afternoon fT levels, may have led to a certain overestimation of the prevalence of hypogonadism. However, diurnal variations are mostly seen in young healthy men and tend to disappear with increasing age [20,21].

The lack of an increase in DHEA in patients developing lipoatrophy under cART has been described previously [22,23]. Christeff *et al.* observed decreasing DHEA levels and an increase in the cortisol:DHEA ratio in patients with aggravating lipodystrophy, whereas an improvement of lipodystrophy was associated with an increase in serum DHEA and a normalization of the cortisol:DHEA ratio [23]. These findings might indicate that DHEA supplementation is beneficial in lipodystrophic HIV-positive men. DHEA treatment in HIV-negative elderly persons has been shown to improve insulin sensitivity and reduce abdominal fat [24]. DHEA has also been reported to have favourable effects on lipodystrophy-associated metabolic changes in HIV-positive patients [25], and might therefore be worth investigating in a randomized controlled trial.

We hypothesize that cART-related insulin resistance provides a pathophysiological link between lipoatrophy and the hormonal changes observed in our patients. Long-standing insulin resistance has been shown to decrease the sensitivity of the HPG axis, resulting in decreased Leydig cell testosterone secretion in men [26]. Also, several observational studies have demonstrated an association of hyperglycaemia and insulin resistance with low DHEA levels [27,28]. Similarly, cART-related insulin resistance could explain the inverse relationship between increasing LH and persistently low fT and DHEA levels in our patients with lipoatrophy. Unfortunately, we could measure neither insulin and cortisol levels nor insulin resistance and thus were not able to investigate the causal link with insulin resistance in this study.

If our hypothesis of a causal link among insulin resistance, lipoatrophy and the observed hormonal changes is valid, a rational intervention would target the metabolic side effects of HIV infection and cART rather than hormonal deficiencies. This might be achieved by choosing ARV regimens with fewer metabolic side effects or by treating metabolic side effects directly. However, trials addressing the effect of glitazones or biguanides [29–34] on lipoatrophy have shown no or limited effects during treatment for up to 1 year.

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Appendix

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