

*Short Communication*

## **In the lipodystrophy associated with highly active antiretroviral therapy, pseudo-Cushing's syndrome is associated with increased regeneration of cortisol by 11 $\beta$ -hydroxysteroid dehydrogenase type 1 in adipose tissue**

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### **Abstract**

*Aims/hypothesis.* Highly active antiretroviral therapy (HAART) in patients infected with human immunodeficiency virus (HIV) is associated with a poorly understood lipodystrophic and hypertriglyceridaemic syndrome, which resembles Cushing's syndrome, but in which plasma cortisol is not elevated. We tested the hypothesis that this HAART-associated lipodystrophy is explained by increased local regeneration of cortisol from inactive cortisone within adipose tissue, catalysed by the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1).

*Methods.* In this cross-sectional study, a previously described cohort of 30 HIV-infected patients with lipodystrophy were compared with 13 HIV-infected patients without lipodystrophy. Intra-abdominal and subcutaneous adipose tissue were quantified using magnetic resonance imaging. Gene expression in subcutaneous fat was measured using real-time PCR. Urine cortisol and its metabolites were analysed by gas chromatography/mass spectrometry.

*Results.* Patients with lipodystrophy had significantly higher 11 $\beta$ -HSD1 mRNA concentrations (relative to  $\beta$ 2-microglobulin mRNA) in subcutaneous adipose tissue than non-lipodystrophic patients ( $0.29 \pm 0.20$  vs  $0.09 \pm 0.07$ ,  $p=0.0004$ ) and higher ratios of urinary cortisol : cortisone metabolites. Adipose tissue 11 $\beta$ -HSD1 mRNA correlated with multiple features of insulin resistance and with mRNA concentrations for glucocorticoid receptor and angiotensinogen.

*Conclusions/interpretation.* In adipose tissue of patients with HAART-associated lipodystrophy, 11 $\beta$ -HSD1 mRNA is increased and its concentration is correlated with features of insulin resistance. We suggest that increased adipose tissue 11 $\beta$ -HSD1 may explain the pseudo-Cushing's features in patients with HAART-associated lipodystrophy, and is a potential therapeutic target.

**Keywords** Adipose tissue · Antiretroviral therapy · Cortisol · Glucocorticoid · HIV · 11 $\beta$ -Hydroxysteroid dehydrogenase type 1 · Lipodystrophy · Real-time PCR

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*Abbreviations:* HAART, highly active antiretroviral therapy · HAL, HAART-associated lipodystrophy · HIV, human immunodeficiency virus · 11 $\beta$ -HSD1, 11 $\beta$ -hydroxysteroid dehydrogenase type 1 · MRI, magnetic resonance imaging · PPAR, peroxisome proliferator-activated receptor

### **Introduction**

Combined highly active antiretroviral therapy (HAART), comprising protease and reverse transcriptase inhibitors, is effective in preventing sequelae of human immunodeficiency virus (HIV) infection. However, some patients taking HAART develop a lipodystrophic syndrome, with loss of subcutaneous fat and accumulation of fat in the abdomen and nape of the neck. This syndrome is associated with hypertriglyceridaemia and insulin resistance [1]. The mechanisms underlying HAART-associated lipodystrophy (HAL) are unknown. The distribution of fat accumulation in

**Table 1.** Characteristics of the study subjects

	Non-lipodystrophic patients (n=13)	Lipodystrophic patients (n=30)	<i>p</i> <sup>a</sup>	Correlation with adipose 11 $\beta$ -HSD1 mRNA	<i>p</i>	Correlation with U cortisol: cortisone metabolite ratio	<i>p</i>
Age (years)	39 $\pm$ 7	43 $\pm$ 10	0.146	0.30	0.063	0.15	0.396
Body mass index (kg/m <sup>2</sup> )	22.4 $\pm$ 3.7	23.6 $\pm$ 2.8	0.128	0.42	0.007	-0.02	0.915
Waist-to-hip ratio	0.89 $\pm$ 0.10	0.99 $\pm$ 0.07	0.003	0.59	<0.0001	0.17	0.325
Systolic blood pressure (mmHg)	121 $\pm$ 10	125 $\pm$ 12	0.308	0.04	0.797	0.14	0.418
Diastolic blood pressure (mmHg)	77 $\pm$ 8	78 $\pm$ 8	0.832	-0.07	0.646	-0.13	0.463
Subcutaneous fat by MRI (cm <sup>3</sup> )	1760 $\pm$ 960	1140 $\pm$ 850	0.040	0.09	0.601	-0.52	0.002
Intra-abdominal fat by MRI (cm <sup>3</sup> )	930 $\pm$ 890	1920 $\pm$ 1110	0.003	0.58	<0.0001	0.28	0.109
Total body fat by bioimpedance (%)	19 $\pm$ 7	17 $\pm$ 7	0.543	0.17	0.298	-0.22	0.210
Plasma glucose (mmol/l)	5.0 $\pm$ 0.5	5.5 $\pm$ 1.5	0.104	0.30	0.064	-0.12	0.516
Serum insulin (mU/l)	6.5 $\pm$ 3.6	11.1 $\pm$ 6.5	0.009	0.51	0.0007	0.18	0.300
Serum triglycerides (mmol/l)	1.2 $\pm$ 0.5	3.4 $\pm$ 2.0	<0.0001	0.58	<0.0001	0.37	0.031
Serum HDL-cholesterol (mmol/l)	1.6 $\pm$ 0.4	1.1 $\pm$ 0.3	0.0004	-0.51	0.0009	-0.44	0.009
Serum total cholesterol (mmol/l)	4.9 $\pm$ 0.7	5.9 $\pm$ 1.1	0.002	0.42	0.008	0.06	0.745
Serum C-reactive protein (mg/l)	0.62 $\pm$ 0.55	1.53 $\pm$ 1.40	0.019	0.43	0.006	0.17	0.347
U cortisol : cortisone metabolite ratio	1.42 $\pm$ 0.21	1.92 $\pm$ 0.62	0.015	0.25	0.173		
Adipose 11 $\beta$ -HSD1 mRNA <sup>b</sup>	0.09 $\pm$ 0.07	0.29 $\pm$ 0.20	0.0004			0.25	0.173

<sup>a</sup> Non-lipodystrophic compared with lipodystrophic patients;

<sup>b</sup> expressed relative to mRNA for  $\beta$ 2-microglobulin; U, urine

HAL is similar to that induced by increased plasma glucocorticoid levels in Cushing's syndrome. However, circulating cortisol concentrations are not elevated in HAL patients [2].

Recent research has highlighted that glucocorticoid action within adipose tissue is regulated independently of circulating cortisol concentrations by the intra-adipose regeneration of cortisol from its inactive metabolite cortisone, catalysed by the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) [3, 4]. Transgenic overexpression of 11 $\beta$ -HSD1 in adipose tissue in mice causes visceral obesity, diabetes and dyslipidaemia [5], while 11 $\beta$ -HSD1 knock-out mice are protected from obesity and stress-related hyperglycaemia [6]. 11 $\beta$ -HSD1 is up-regulated by inflammatory cytokines [7].

Here, we test the hypothesis that increased adipose tissue 11 $\beta$ -HSD1 is associated with lipodystrophy and its metabolic manifestations in a previously described cohort of HIV-seropositive patients taking HAART [8, 9].

## Subjects and methods

All participants were attending an HIV outpatient clinic, had been taking HAART for at least 18 months, and gave written consent for the study, which was approved by the local ethics committee. We studied 5 women and 25 men who reported lipodystrophic symptoms and had clinical features of lipodystrophy confirmed by one investigator. As control subjects we studied four women and nine men without symptoms or clinical features of HAL. None of the women was postmenopausal and the age distribution was almost identical between the groups

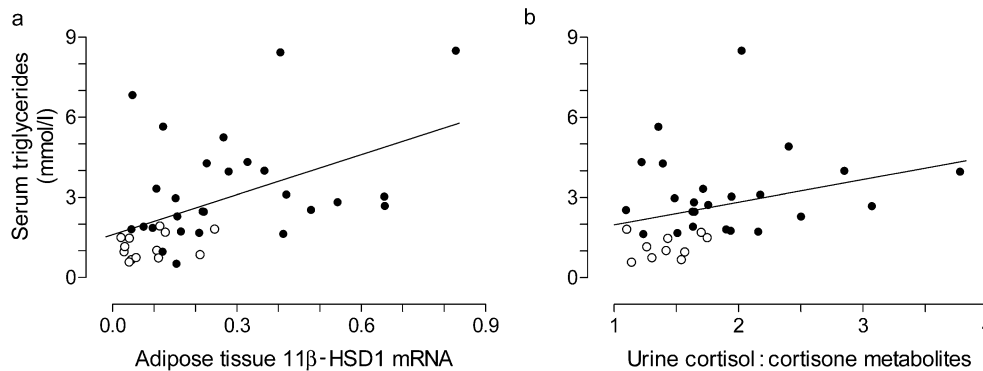
(38 $\pm$ 4 vs 37 $\pm$ 6 years, lipodystrophic vs non-lipodystrophic subjects). Patients with diabetes mellitus were excluded.

Body fat and its distribution were assessed by body mass index, waist-to-hip ratio, bioimpedance and cross-sectional abdominal magnetic resonance imaging (MRI) scans. Blood was obtained at 08.00 hours after an overnight fast for glucose, insulin, lipid and C-reactive protein analyses. Thereafter, a needle aspiration biopsy of abdominal subcutaneous fat was obtained, from which RNA was extracted and mRNA species quantified by real-time polymerase chain reaction in 40 participants (13 non-lipodystrophic) [8, 9, 10]. The mRNA results are expressed relative to mRNA for  $\beta$ 2-microglobulin. Early-morning urine was collected from 35 (10 non-lipodystrophic) participants for analysis of cortisol and its metabolites by gas chromatography/mass spectrometry [10].

The Mann-Whitney *U* test was used to compare differences between the groups. Correlations were calculated using Spearman's rank correlation coefficient. All data are given as means  $\pm$  SD. A *p* value of less than 0.05 was considered statistically significant.

## Results

As described previously [8, 9], the combinations of HAART drugs and severity or duration of HIV-1 infection did not differ between HAL and non-HAL patients. The most common non-HIV medications being taken were co-trimoxazole for prophylaxis for *Pneumocystis carinii* pneumonia (20% vs 15%, lipodystrophic vs non-lipodystrophic group) and pravastatin (10% vs 8%, respectively). Use of other drugs was infrequent (0–1 patients in either group used acyclovir, cetirizine, loperamide, mirtazapine, omeprazole) with no difference between the groups.



**Fig. 1.** Prediction of severity of hypertriglyceridaemia by (a) adipose 11 $\beta$ -HSD1 mRNA (expressed relative to mRNA for  $\beta$ 2-microglobulin) and (b) urinary cortisol : cortisone metabolites, i.e. the ratio of (5 $\alpha$ - + 5 $\beta$ -tetrahydrocortisol) : tetrahydrocortisone. Filled symbols: patients with lipodystrophy; open symbols: patients without lipodystrophy. For comparisons between groups and Spearman rank correlations, see Table 1

Patients with HAL had higher intra-abdominal fat volume, waist-to-hip ratio, fasting serum insulin, triglycerides and C-reactive protein, and lower HDL-cholesterol (Table 1). There were no differences in blood pressure, body mass index or total % body fat, but the abdominal subcutaneous fat volume was reduced in HAL patients. In subcutaneous adipose tissue biopsies, patients with HAL had markedly higher mRNA levels for 11 $\beta$ -HSD1 (Table 1). In all subjects, adipose tissue 11 $\beta$ -HSD1 mRNA correlated positively with visceral fat volume, waist-to-hip ratio, body mass index, fasting serum insulin, triglycerides (Fig. 1a), total cholesterol and C-reactive protein, and correlated inversely with HDL-cholesterol. However, adipose tissue 11 $\beta$ -HSD1 mRNA did not correlate with subcutaneous fat volume or with total % body fat (Table 1).

In urine, patients with HAL had higher ratios of cortisol : cortisone metabolites (Table 1). The urine cortisol : cortisone metabolite ratio correlated positively with fasting serum triglycerides (Fig. 1b) and inversely with subcutaneous fat volume and HDL-cholesterol (Table 1). Sex and age had no confounding effects on adipose tissue 11 $\beta$ -HSD1 mRNA or on urinary cortisol : cortisone metabolite ratios.

To assess the impact of increased 11 $\beta$ -HSD1 on intra-adipose tissue glucocorticoid signalling in HAL patients, other adipose tissue mRNAs were also quantified. Levels of 11 $\beta$ -HSD1 mRNA were positively correlated with mRNA levels for the glucocorticoid receptor (Spearman  $r=0.43$ ,  $p=0.006$ ) and for the glucocorticoid-sensitive target gene angiotensinogen ( $r=0.35$ ,  $p=0.031$ ). For other genes involved in regulation of adipose tissue metabolism and hormonal signalling mRNAs have been quantified previously in this cohort [8, 9]. Interestingly, 11 $\beta$ -HSD1 mRNA levels were correlated positively with mRNA levels

for TNF- $\alpha$  ( $r=0.64$ ,  $p<0.0001$ ) and inversely with mRNA levels for leptin ( $r=-0.38$ ,  $p=0.015$ ) and peroxisome proliferator-activated (PPAR $\gamma$ ) ( $r=-0.55$ ,  $p=0.0003$ ); leptin and cytokines up-regulate, and PPAR $\gamma$  agonists (thiazolidinediones) down-regulate 11 $\beta$ -HSD1 in cells.

## Discussion

We conclude that 11 $\beta$ -HSD1 mRNA is increased in adipose tissue of patients with HAL and correlates with the severity of intra-abdominal fat accumulation and metabolic disturbances. The increased ratio of cortisol : cortisone metabolites in urine supports the inference that in vivo conversion of cortisone to cortisol by 11 $\beta$ -HSD1 is enhanced in these patients. The associated increase in mRNA for glucocorticoid receptor and angiotensinogen suggests that increased 11 $\beta$ -HSD1 mRNA increases intra-adipose glucocorticoid signalling and could account for the pseudo-Cushing's characteristics of patients with HAL.

The mechanism that leads to increased adipose tissue 11 $\beta$ -HSD1 in HAL is unclear. In cells in culture, 11 $\beta$ -HSD1 is regulated by metabolic and inflammatory stimuli. Although the relevance of these observations in vivo in humans remains to be established, the associations observed by us in this study suggest that increased cytokine activity and decreased PPAR $\gamma$  activation may be important. Protease inhibitors, with which lipodystrophy was originally associated, have been shown to inhibit rather than enhance 11 $\beta$ -HSD1 activity in 3T3-L1 adipocytes [7].

Levels of 11 $\beta$ -HSD1 are also greater in subcutaneous adipose tissue from obese subjects than in that from non-obese subjects [10]. Notably, this is associated with increased rather than decreased leptin expression, and does not seem to be accompanied by increased glucocorticoid receptor or angiotensinogen expression [10]. The findings in HAL patients appear to more closely mirror those in Cushing's syndrome and in mice with adipose tissue 11 $\beta$ -HSD1 overexpression than those in idiopathic obesity. Although not directly addressed here, a possible explanation is that glucocorticoid receptor activation is increased both in subcutaneous and in omental adipose tissue in Cush-

ing's syndrome, in mice overexpressing 11 $\beta$ -HSD1 and in HAL, whereas in idiopathic obesity glucocorticoid receptor activation is only increased in subcutaneous adipose tissue.

These observations have important clinical implications. Polymorphisms that influence 11 $\beta$ -HSD1 expression may underlie inter-individual differences in susceptibility to HAL and may form the basis for pharmacogenetic strategies in future. Currently, 11 $\beta$ -HSD1 inhibitors are being developed which, if active in adipose tissue, may offer a novel approach to prevent or treat visceral fat accumulation and insulin resistance in HAART-treated patients.

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## References

1. Carr A, Cooper DA (2000) Adverse effects of antiretroviral therapy. *Lancet* 356:1423–1430
2. Miller KK, Daly PA, Sentonick D et al. (1998) Pseudo-Cushing's syndrome in human immunodeficiency virus-infected patients. *Clin Infect Dis* 27:68–72
3. Seckl JR, Walker BR (2001) Minireview: 11beta-hydroxysteroid dehydrogenase type 1—a tissue-specific amplifier of glucocorticoid action. *Endocrinology* 142:1371–1376
4. Stulnig TM, Waldhäusl W (2004) 11 $\beta$ -Hydroxysteroid dehydrogenase type 1 in obesity and type 2 diabetes. *Diabetologia* 47:1–11
5. Masuzaki H, Paterson J, Shinyama H et al. (2001) A transgenic model of visceral obesity and the metabolic syndrome. *Science* 294:2166–2170
6. Kotelevtsev Y, Holmes MC, Burchell A et al. (1997) 11beta-Hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. *Proc Natl Acad Sci USA* 94:14924–14929
7. Tomlinson JW, Moore J, Cooper MS et al. (2001) Regulation of expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue: tissue-specific induction by cytokines. *Endocrinology* 142:1982–1989
8. Kannisto K, Sutinen J, Korshennikova E et al. (2003) Expression of adipogenic transcription factors, peroxisome proliferator-activated receptor gamma co-activator 1, IL-6 and CD45 in subcutaneous adipose tissue in lipodystrophy associated with highly active antiretroviral therapy. *AIDS* 17:1753–1762
9. Yki-Järvinen H, Sutinen J, Silveira A et al. (2003) Regulation of plasma PAI-1 concentrations in HAART-associated lipodystrophy during rosiglitazone therapy. *Arterioscler Thromb Vasc Biol* 23:688–694
10. Wake DJ, Rask E, Livingstone DE, Soderberg S, Olsson T, Walker BR (2003) Local and systemic impact of transcriptional up-regulation of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue in human obesity. *J Clin Endocrinol Metab* 88:3983–3988