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RESEARCH LETTER

Cushingoid lipodystrophy can be prevented by thiazolidinediones

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Introduction

The lipodystrophy, frequently observed with prolonged hypercortisolism (i.e., Cushing's disease), is characterized by increased visceral adipose tissue (VAT) and decreased peripheral subcutaneous adipose tissue (SAT). A very similar lipodystrophy is seen in HIV patients, particularly when they are using protease inhibitors [1]. Although not demonstrated, it has been suggested that this cushingoid lipodystrophy results from differences in insulin resistance between VAT and SAT. However, cortisol also affects lipid metabolism through glyceroneogenesis (GLN), wherein non-glucose substrates such as lactate, pyruvate, and amino acids are converted to glycerol to form triacylglycerol. Regulation of GLN mainly occurs via expression of the enzyme phosphoenolpyruvate-carboxykinase (PEPCK). Specifically, cortisol increases PEPCK expression in the liver, while in adipose tissue induces the opposite effect. Thus, it is possible that the cushingoid lipodystrophy results from alterations in GLN [2]. In addition, the lipodystrophy of HIV has also been shown to be mediated by glyceroneogenesis [3].

It has been shown that in diabetic patients thiazolidinediones [TZDs; agonists of the peroxisome proliferator-activated receptor gamma (PPAR-γ) in adipose tissue] increase SAT while decrease VAT [4], and that these TZD's effects occur via GLN modulation [5]. In addition, it has been proposed that the cushingoid habitus is explained by selective expression of 11-β-hydroxysteroid dehydrogenase type 1 (11-β-HSD-1) in VAT [6], and this enzyme is apparently inhibited by TZD's in adipose tissue [7]. Consequently, the hypothesis that the TZD rosiglitazone prevents cushingoid lipodystrophy was tested in this study. To this end, a modified version of an animal model of Cushing's disease was used [8].

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Methods

Adult female mice (C57/BL6, $n = 31$) were singly housed and divided into three groups. The first group, CONTROL ($n = 11$) had ad libitum access to water containing 1 % ethanol vehicle (vehicle). The second group, CORT ($n = 10$) was given an aqueous solution of 100 μg/ml hydrocortisone (Sigma-Aldrich, MO) in 1 % ethanol for 24 days. Although the original model entailed ad libitum access to this solution [8], an 80 % mortality rate was observed when we used this regimen. To avoid such a lethal hydrocortisone intake due to polydipsia, the hydrocortisone concentration was adjusted by the daily water consumption to keep the hydrocortisone intake between 0.5 and 1.0 mg/day. The third group, CORT + TZD ($n = 10$) was given the same hydrocortisone regimen as the CORT group, but with added daily gavage of 0.2 ml of rosiglitazone ($\sim$5 mg/kg/day). All mice had ad libitum access to standard rodent chow. Weights, fluid consumption, and activity levels were monitored daily. After 24 days, animals were euthanized and dissected. Weights of the liver, adrenals, and VAT (intraperitoneal, peri-gonadal, and perinephric adipose in aggregate) were measured. Pooled data are shown as mean ± SD. Statistical differences, calculated by one-way analysis of variance (ANOVA), were considered significant when p values were <0.05. The Bonferroni t test was applied to all multiple-group comparison analyses.

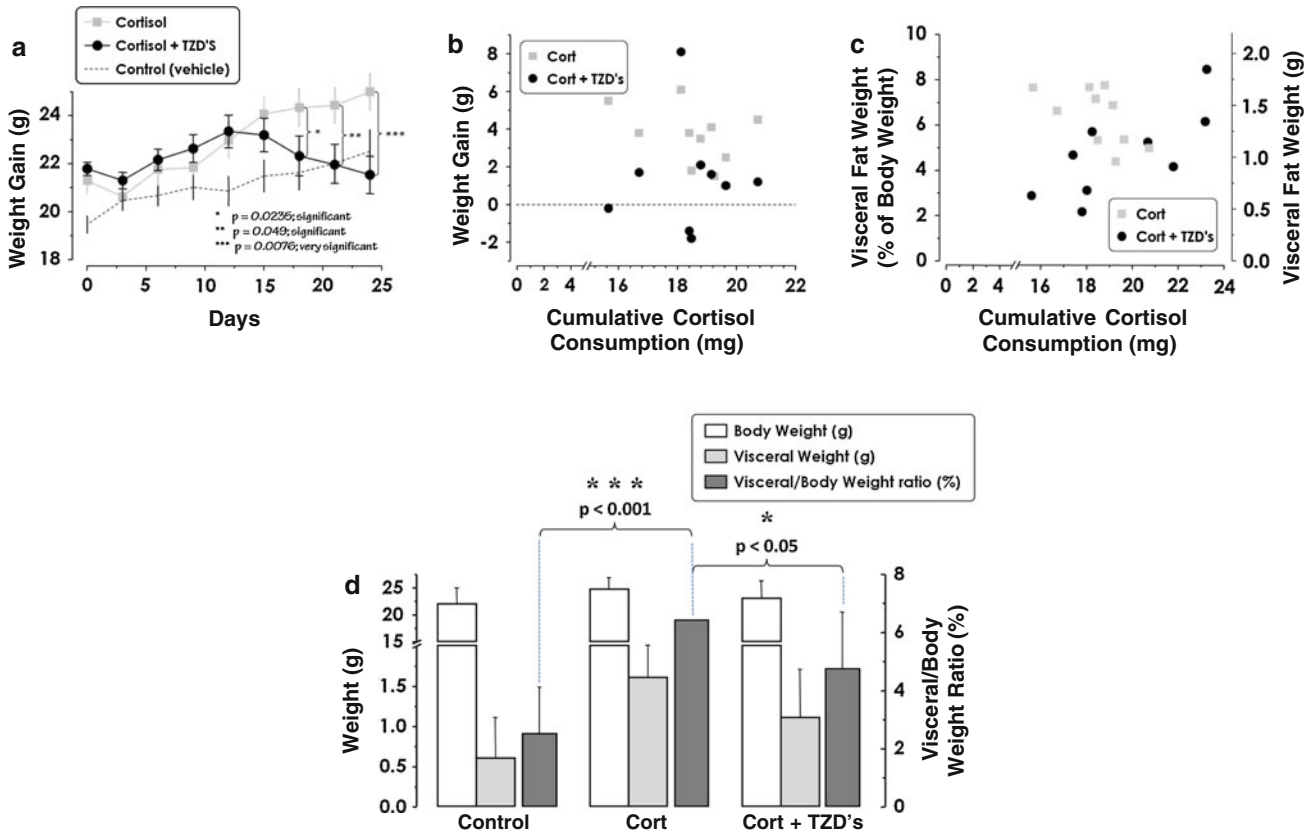

Fig. 1 **a** Total body weight of the control (*dashed*; $n = 11$), cortisol (*light gray*; $n = 10$), and cortisol + TZD's (*black*; $n = 10$) mice, throughout the duration of the study (24 days). Statistical significance of cortisol versus cortisol + TZD's tested at 18, 21, and 24 days. **b** Averaged total body weight gain as a function of cumulative cortisol consumption of the cortisol (*light gray*) and cortisol + TZD's (*black*) groups (standard deviations not shown). **c** Averaged visceral fat with (as *percent left axis*; in *g right axis*) as a function of cumulative cortisol consumption of the cortisol (*light gray*) and cortisol + TZD's (*black*) groups (standard deviations not shown). **d** Pooled data of body and visceral weights and visceral/body weight ratio after 24 days under control, cortisol, and cortisol + TZD's regimens. Statistical significance of visceral/body weight ratio tested for control versus cortisol and for cortisol versus cortisol + TZD's

All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC #2061).

Results

The daily hydrocortisone consumption of CORT and CORT + TZD groups was about 0.7 mg/day ($\sim$30 mg/kg/day), without showing significant differences ($p = 0.74$). In both groups, there was no association between cumulative cortisol consumed and weight gained or lipodystrophy. Adrenal mass, a marker of adrenal suppression, was also significantly reduced in the CORT group compared with the control group (16 ± 6 vs. 5 ± 2 mg, respectively; $p = 4.7 \times 10^{-5}$). VAT was significantly larger in the CORT than in the CONTROL groups, both in net weight (1.6 ± 0.1 vs. 0.6 ± 0.5 g) as well as body weight percent (6.4 vs. 2.5 %, respectively; $p = 6 \times 10^{-6}$). VAT however, was significantly lower in the CORT + TZD group than in the CORT group (1.1 ± 0.6 vs. 1.6 ± 0.1 g, respectively; or 6.4 vs. 4.7 %, respectively; $p = 0.04$). While the overall weight gain was not significantly different between the CORT and CONTOL groups (3.0 and 3.7 g, respectively; $p = 0.52$), the CORT + TZD group gained less weight than the CORT group (1.1 vs. 3.7 g, respectively; $p = 0.02$). Interestingly, the CORT + TZD group gained even less weight than the CONTROL group (1.1 vs. 3.0 g, respectively; $p = 0.04$).

An intriguing finding was that lipodystrophy (i.e., visceral/body ratio) in the CORT group was inversely proportional to cumulative cortisol consumption; while in the CORT + TZD group this relationship was reversed (data not shown).

The mean liver mass in the CORT, CORT + TZD, and CONT groups were 1.6 ± 0.1, 1.2 ± 0.1, and 1.5 ± 0.2 g, respectively. The livers of the CORT + TZD group were significantly smaller than the CORT group ($p = 4 \times 10^{-6}$), while the CORT and CONT groups were not significantly different with respect to liver mass. Notably, the livers of the CORT group appeared very greasy, in contrast to the livers of the other groups.

Discussion

Our data indicate that TZD's significantly reduced the gain in body weight gain as well as the visceral fat accumulation typically associated with hypercortisolism. This suggests that TZDs can prevent cushingoid lipodystrophy, possibly by GLN modulation through PEPCK activity and/or by affecting 11-β-HSD-1 expression in VAT.

The effectiveness of our experimental model to induce Cushing's syndrome was supported by substantial changes in body weight and fat distribution, and demonstrated by a significant reduction of adrenal mass; which indicates pituitary corticotropin depression induced by the high levels of exogenous cortisol.

Liver mass was examined because TZD could be reversing cortisol's action on GLN in this organ. Although TZD's stimulate PPAR-γ and this is not abundant in the liver, the TZD pioglitazone has been shown useful in the treatment of hepatic steatosis associated with diabetes [9]. Our finding that the livers from the CORT group appeared noticeably greasier than those from the CORT + TZD group supports the assumption that the difference in hepatic weight is related to the lipid content.

The results of this study need to be expanded to define: (a) the TZD's effects on long-term hydrocortisone exposure, (b) the specific mechanism(s) of action of TZD's on cushingoid lipodystrophy, and (c) the inversely proportional relationship between lipodystrophy and consumed cortisol, and why this relationship was reversed with the addition of the TZD. TZD's action on chronic hypercortisolism is particularly intriguing in light of our observation that after the second week of TZD administration body weight reached values below the control (Fig. 1a). In terms of clinical applications of our work, it should be mentioned that pioglitazone has a much safer side effect profile than rosiglitazone and at least in theory, it would have no differences in the amelioration of lipodystrophy investigated here. Metformin is currently the preferred drug for treating the diabetes due to hypercortisolism and it has also been shown to be effective in reducing the lipodystrophy of HIV [10, 11]. Consequently, a comparative study of the efficacy of TZDs and metformin in reducing the lipodystrophy of hypercortisolism would be valuable.

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