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Oleoyl-estrone increases adrenal corticosteroid synthesis gene expression in overweight male rats

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ABSTRACT

Oleoyl-estrone (OE) induces a marked loss of body fat in rats by maintaining energy expenditure, body protein and blood glucose despite decreasing food intake. OE increases glucocorticoids, but they arrest OE lipid-mobilization. We studied here whether OE induces a direct effect on adrenal glands function as part of this feedback regulation. Dietary overweight male rats were given oral 10 nmol/g OE gavages for ten days. A group (PF) of pair-fed to OE rats, and controls received vehicle-only gavages. OE rats lost slightly more body than PF, but had larger adrenal glands. Tissue corticosterone levels, and gene expressions for glucocorticoid-synthesizing enzymes were increased in OE versus controls and PF; thus, we assumed that adrenal growth affected essentially its cortex since OE also lowered the expression of the medullar catecholamine synthesis enzyme genes. Serum corticosterone was higher in PF than in OE and controls, but liver expression of corticosteroid-disposing steroid 5α-reductase was 3× larger in OE than PF and controls. Circulating glucocorticoids changed little under OE, in spite of higher adrenal gland and liver content, hinting at modulation of glucocorticoid turnover as instrumental in their purported increased activity. In conclusion, we have observed that OE considerable enhanced the expression of the genes controlling the synthesis of glucocorticoids from cholesterol in the rat and increasing the adrenal glands' corticosterone, size and cellularity, but also the liver disposal of corticosteroids, suggesting that OE increases corticosterone synthesis and degradation (i.e. serum turnover), a process not driven by limited energy availability but directly related to the administration of OE.

1. Introduction

Glucocorticoids play a key role in the body response to aggression, largely controlling the hystic response to extraneous or potentially dangerous compounds and restoring the alterations induced by the body responses to insult, exercise or harm [1]. The soothing effect of glucocorticoids, i.e. after a catecholamine-driven muscular exercise, largely consists in: controlling the immune system responses, restoring the energy metabolism parameters and activate the reparation of damages [2]. Stress, continuing dietary aggression and other prolonged situations eliciting a glucocorticoid response may result in overstretching their corrective function, depressing a number of paths below normalcy: altered circadian rhythms [3], appetite [4] and sleep [5], depression [6], deregulation of inflammatory [7] and immune [8] responses and a derangement of the energy homeostasis, starting with insulin resistance [9] and continuing with severe accumulation of fat in the blood, liver, muscle and adipose tissue [10], which starts the cascade of counteractions that generate the multiple facets of the metabolic syndrome [11].

The metabolic syndrome, and obesity as one of its main pathological manifestations is directly related to inflammation [12]. It is widely accepted that the alteration of adipose tissue endothelial function and signalling may be the consequence of long-term or sustained excess nutrient availability [13]; this results in a continued saturation of the adipose tissue capability to store fat that elicits a cytokine-driven response [14,15] that, in the long-term, results in additional alteration of energy metabolism and homeostasis [16]. The basis of the tissue insult lies on the endothelium, which inflammation (involving active radicals of oxygen and nitrogen) [12,17] starts a response cascade in which the counteractive actions of adipokines, cytokines and other hormonal signals, both peptidic and steroidal acquire a protagonist role [17,18]. The removal of the

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adrenal gland prevents or even reverses obesity in several animal models [19,20] enhancing the slimming effects of leptin [21] and oleoyl-estrone [22].

Oleoyl-estrone (OE) is a powerful slimming agent [23] that induces an energy imbalance [24] by decreasing food intake and maintaining energy expenditure [25]. The action of OE results in the maintenance of body protein [23,24] and glycemia as well as liver glycogen [26]. But muscle utilization of lipid in obese rats is enhanced in parallel to the normalization of circulating lipids and the wasting of overall body fat [27]. Oral OE administration does not produce estrogenic effects [28], in spite of the considerable release of estrone by tissue OE hydrolysis [29], because conversion of estrone into estradiol is limited by OE itself via inhibition of 17β-hydroxysteroid dehydrogenase [30]. The administration of estrone alone does not induce the slimming effects of OE either [31]; the effects of OE, thus, could not be explained by estrogenic effects, irrespective of the limited increase in circulating estrogen induced by OE treatment [32]: OE is a ponderostat signal [33] rather than a simple estrogen-carrier molecule.

OE increases tissue glucocorticoid availability in female rats by (a) increasing hypothalamic CRH, plasma corticotropin and circulating corticosterone [34,35], (b) decreasing plasma binding, thus enlarging the free fraction of corticosterone [36], and (c) increasing the expression and activity of 11β-hydroxysteroid dehydrogenase in the liver of adrenalectomized rats, even after compensatory corticosterone administration [37].

Corticosterone administration inhibits the lipid-mobilizing action of OE in adrenalectomized rats [38]. The overall effects of OE on energy metabolism (including insulin resistance) are fairly opposed to those of glucocorticoids, which may suggest that the rise in corticosteroids may be a reaction to the energy-wasting counteractive action of OE.

In the present study we intended to check whether the effects of OE on the circulating glucocorticoids of overweight (but otherwise normal) rats are a consequence of altered adrenal function. We included a pair-fed group in the study as a way to discern whether the effects observed were due to OE action or to the decreased energy intake consequence of this treatment.

2. Experimental

2.1. Animals and sample preparation

Adult male Wistar rats were made overweight by a limited period of cafeteria diet feeding, as previously described [39]. The rats, initially weighing 355 ± 5 g, were kept under standard conditions of housing and feeding [39]. Three groups of 8 rats each were randomly selected: controls, OE and 'pair-fed' (PF). All animals received every day an oral gavage of 0.2 mL of sunflower oil (7 kJ), which was supplemented in the OE group with 10 nmol/g oleoyl-estrone (OED, Barcelona, Spain). The controls and OE group had free access to pellet food (maintenance chow, Panlab, Barcelona, Spain), and the PF rats were allowed to eat only the mean food consumption on the matching day of the OE group; all rats had water available *ad libitum*. On day 10, the rats were killed by decapitation and the adrenal glands, and liver were dissected, weighed, frozen in liquid nitrogen and kept at −80 °C. Blood samples were allowed to clot and serum was also stored at −80 °C until processed.

The animals were kept, handled and killed following the specific procedures approved by the University of Barcelona Animal Welfare and Ethics Committee, in full conformity with the norms and proceedings set forth by the European Union and the Governments of Spain and Catalonia.

2.2. Serum steroid hormone analysis

Aliquots (0.400 mL) of rat serum were mixed with 0.025 mL of a working stock mixture of d8-corticosterone (1×10^{-5} M), added as an internal standard, and the tubes were incubated on ice for 20 min. Acetonitrile (1.5 mL) was then added and the tubes were vortexed and centrifuged to remove proteins. Supernatants were transferred into glass tubes and evaporated to dryness under nitrogen; dried residues were redissolved in 0.120 mL of the mobile phase.

Sample analyses were performed by LC–MS/MS (Varian, Palo Alto, CA, USA) consisting of a 210 pump with an online degassifier, a 410 autosampler, and a 1200 L triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operated in positive ion mode. The chromatographic separation was performed in a 150 × 3.0 (i.d.) mm Intersil 3 ODS-3 column (Varian, Palo Alto, CA, USA). The mobile phase consisted of two eluents, solvent A (5 mM ammonium acetate, 0.1% formic acid) and solvent B (acetonitrile), at a flow rate of 0.2 mL/min. Samples of 0.050 mL were injected into the LC–MS/MS, after which the injector was washed five times with mobile phase. The total run time was 55 min. A linear gradient from 30% to 55% B was programmed for the first 28 min, followed by a second linear gradient to 90% B for 4 min, and isocratic mode for the next 16 min. Then, the system was returned to the initial proportion of 30% solvent B over the following 2 min and maintained for the final 5 min of each run.

Deuterated steroids were purchased from CDN Isotopes Inc (Pointe Claire, Canada). All other steroid standards were from Sigma (St Louis, MO, USA). Calibration curves were prepared in the range 1–4000 nM. The ratio between the peak area of each endogenous steroid and the deuterated steroid (d8-corticosterone) was correlated with the concentration using linear regression analysis to quantify serum steroid levels. Detection limits of the method were 2 nM for dehydrocorticosterone and 10 nM for corticosterone.

Serum corticosterone binding was carried out as previously described [40] using ^{3}H-corticosterone (NET399, PerkinElmer, Boston, MA, USA).

2.3. Tissue corticosterone analysis

Frozen adrenal glands and liver samples were homogenized, in about 10 volumes of chilled 0.3 N perchloric acid containing 9 g/L NaCl [41], using a Polytron (Kinematica, Luzern, Switzerland) homogenizer. After centrifugation, clear supernantants were diluted (1/3 liver, 1/400 adrenals) and used for a corticosterone radioimmunoanalysis (MP Biomedical, Oranburg, NY, USA) [42]. The data were expressed per unit of tissue weight.

2.4. Nucleic acid analyses, cellularity

Tissue samples were used for the estimation of total DNA, using a standard fluorimetric method with 3,5-diaminobenzoic acid (Sigma, St Louis, MO, USA) and bovine DNA (Sigma) as standard [43]. Tissue DNA content allowed the calculation of the number of cells per gram of tissue and in the whole tissues sampled, based on the assumption that the DNA content per cell is constant in mammals; here we used the genomic DNA size data [44] for somatic rat cells (5.6 pg/cell). Mean cell mass was estimated from the number of cells and the weight of the organ.

Total tissue RNA was extracted using the Tripure reagent (Roche Applied Science, Indianapolis IN USA), and were quantified in a ND-100 spectrophotometer (Nanodrop Technologies, Wilmington DE USA).