OLEOYL-ESTRONE METABOLIC EFFECTS IN RELATION WITH CALORIC RESTRICTION IN INBRED BETA RATS WITH SPONTANEOUS OBESITY AND TYPE 2 DIABETES

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Abstract
Spontaneously hypertriacylglycerolemic obese and diabetic inbred IIM Beta rats were treated with oleoyl-estrone for 10 days. Pair-feeding was performed to determine some oleoyl-estrone effects dependent on the caloric restriction it promotes. Twenty-five 200 day-old Beta males receiving a daily gavage of 0.2 ml sunflower oil were divided into the following groups: 1) daily dose of 10 nmol/g oleoyl-estrone; 2) pair-fed; 3) control. The variables measured were: whole body protein, water and lipid; retroperitoneal and epididymal fat depot weights; plasma urea, glucose, insulin, triacylglycerols and cholesterol. Biomass and food intake were assessed daily. Oleoyl-estrone and pair-fed groups expressed similar variations in body composition and significant body weight losses due to reduction in food intake. Oleoyl-estrone and pair-fed treatments significantly reduced retroperitoneal fat depot weights, but not epididymal ones. In oleoyl-estrone and pair-fed groups hyperglycemia decreased and insulinemia lowered significantly. Plasma normal total cholesterolemia and hypertriacylglycerolemia values typical of Beta rats decreased strongly compared to controls, though attaining significantly different values between oleoyl-estrone and pair-fed groups. Plasma total cholesterol appeared as more sensitive to caloric restriction than triacylglycerols through a specific oleoyl-estrone-mediated effect.

Key words: oleoyl-estrone, inbred Beta rats, obesity, slimming, diabetes

Oleoyl-estrone (OE) has been reported as one fatty acyl ester of estrone carried by lipoproteins with an important function in the signaling system of energy homeostasis. It has been suggested that OE does not act through leptin, but that these hormones share a close functional relationship. Chronic administration of OE either i.v. in liposomes or orally, to lean or obese rodents, results in a progressive loss of fat stores due mainly to lower caloric intake while energy expenditure is maintained. Rodents on OE treatment lose weight without any apparent side effect. It has been demonstrated that the presence of OE in the diet promotes a loss of appetite not directly mediated by NPY (neuropeptide Y).
CRH (corticotropin releasing hormone) without taste aversion side effects. OE produces alterations in the plasmatic lipidic profile in both lean and obese rats (lowered plasma total cholesterol as well as other types of lipids in rats). Moreover, in OE treated rats variations of internal (e.g. retroperitoneal) white adipose tissue (WAT) depots were larger than those of external (e.g. epididymal) ones. Treatment with OE has been described to affect body protein less intensively than hypocaloric dieting alone (6) but no comparison with pair-fed animals has been reported.

Inbred IIM Beta (Beta) strain of rats, from the IIM stock, is a suitable model for human hypertriacylglycerolemic mild obesity and progressive glucose intolerance evolving towards type 2 diabetes.

The aim of this study was to describe the effects of OE on spontaneously obese and diabetic Beta rats as well as to determine OE effects dependent on the caloric restriction it promotes by comparing OE treated rats to pair-fed ones.

Materials and Methods

Rats, feeding and oleoyl-estrone

IIM Beta (Beta) strain of rats form IIM stock has been inbred by a non-regular system using small populations. Twenty-five 200 day-old male Beta rats with well developed obesity and diabetes, were allocated in individual cages four days before the beginning of the treatments. They were kept under standard lighting (12h light-12h dark), temperature (21 ± 2°C) and relative humidity (60-75%), and were fed standard rodent chow pellets (Cargill®). The composition of this feed in g/100 g was: moisture: 10.9; crude protein (f: 6.25) 23.9; lipids: 5.5; fiber: 8.2; digestible carbohydrates: 45.2; minerals: 6.3. The energy equivalence, after correcting for digestibility was 1335.9 KJ/100g. The mean ad libitum daily intake before the beginning of the treatments was 28 ± 4g = 374 ± 53 KJ. Additional 7.5 KJ from the sunflower oil given by stomach probe were daily added during the experiment.

Oleoyl-estrone was synthesized from oleoyl-chloride and estrone in an anhydrous pyridine medium by M. Alemany (Departament de Bioquimica I Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain).

Experimental design

Three groups of Beta 200 days-old male rats were randomly selected for the following treatments: 1- (OE): fed ad libitum plus an oral daily dose of 10 nmol/g body weight of oleoyl-estrone (n=10). 2- (PF): pair-fed to OE (n=7). This group was assumed equivalent to those of controls on day 11, assuming the lack of variation over ten days in this adult age.

Rats were sacrificed by ether overdose on day 11 after the beginning of treatments and blood was collected by partial decapitation. Hair and gastrointestinal content were removed. Epididymal and retroperitoneal fat pads were dissected, weighed and restored to the rest of the carcass which included skin and subcutaneous fat depot. This was weighed and stored at −20°C until processing. The frozen carcass was crushed and minced until homogenized. Duplicate aliquots from every rat were used for the following analyses:

- **Moisture:** estimated by drying at 102-105°C until weight loss in 1 h ≤ 1mg. (AOAC 1990, method 950.46)
- **Fat content:** determined on the dried samples with chloroform/methanol 50/50 ( Folch extractant) in a discontinuous Soxhlet extractor.
- **Crude protein:** calculated from oleoyl-chloride and estrone in an anhydrous pyridine medium by M. Alemany.
- **Energy equivalence:** after correcting for digestibility was 1335.9 KJ/100g.

Glycemia: determined by an enzymatic method using a commercial kit (Wiener Lab, Argentina).

Uremia: determined by a specific enzymatic method for blood and urine urea determination with a commercial kit (Wiener Lab, Argentina).
**Cholesterolemia**: cholesterol was determined by an enzymatic method using a commercial kit (Wiener Lab, Argentina)\(^b\).

**Triacylglycerolemia**: determined by an enzymatic Trinder colorimetric method for serum or plasma triacylglycerols determination with a commercial kit (Wiener Lab, Argentina)\(^b\).

**Insulinemia**: quantified by a solid-phase \(^1\)\(^2\) radioimmunoassay designed for the quantitative measurement of insulin in serum using a commercial kit (Coat-A-Count Insulin-Diagnostic Products Corporation, USA)\(^b\).

**Statistics**

Data are presented as means ± standard error (SEM) and have been compared using ANOVA and two-tailed \(t\) test for unpaired data. Mean values were considered significantly different for \(p \leq 0.05\).

**Results**

OE promoted a reduction in food intake. The largest reductions in OE as well as in PF were from day 4 to day 7 (Fig. 1). Total food—and caloric intake—were significantly higher in CO: 278 ± 14 g (3713.8 ± 187 kJ) compared with OE: 155 ± 10 g (2070.6 ± 134 kJ) or PF: 149 ± 5 g (1990.5 ± 67 kJ) (\(p < 0.001\)).

The average initial weight of the rats was 436.28 ± 8.51 g. Treatment with OE as well as PF resulted in a body weight loss of about 10% on day 11, while CO gained about 2% of their initial biomass (Fig. 2).

Table 1 summarizes the variations of biomass as well as the body amounts of protein, water and lipid following treatments. Amounts on day 0 were assumed alike for all treatments.

| TABLE 1.— Biomass and body protein, water and lipid in 200 days-old male Beta rats with mild obesity and type 2 diabetes treated with oleoyl-estrone or pair feeding |
|---|---|---|---|---|---|---|
| | Relative weight (g/100 g) | | | Weight (g) | |
| | O. Estrone (n=6) | Pair-fed (n=7) | Control (n=5) | O. Estrone (n=6) | Pair-fed (n=7) | Control (n=5) |
| Biomass day 0 | | | | | | |
| Biomass day 11 | 100 | 100 | 100 | \(428.7 \pm 21.5\) \(c\) | 401.4 ± 11.3 \(c\) | 426.4 ± 28.5 \(c\) |
| Protein Estimation day 0 | 2 14.3 | 14.3 | 14.3 | \(57.2 \pm 18.2\) \(cd\) | 362.7 ± 9.7 \(c\) | 432.8 ± 24.6 \(d\) |
| Protein Content day 11 | 4 15.2 ± 0.6 \(a\) | 14.8 ± 0.1 \(a\) | 14.3 ± 0.1 \(b\) | \(57.1 \pm 2.5\) \(c\) | 53.6 ± 1.4 \(c\) | 62.0 ± 3.7 \(d\) |
| Water Estimation day 0 | 2 60.7 | 60.7 | 60.7 | \(260.2 \pm 9.9\) \(cd\) | 225.8 ± 6.8 \(c\) | 262.5 ± 13.5 \(d\) |
| Water Content day 11 | 4 61.8 ± 0.6 \(a\) | 62.2 ± 0.8 \(a\) | 60.7 ± 0.4 \(a\) | \(232.9 \pm 9.9\) \(cd\) | 225.8 ± 6.8 \(c\) | 262.5 ± 13.5 \(d\) |
| Lipid Estimation day 0 | 2 19.9 | 19.9 | 19.9 | \(85.3 \pm 3\) | 79.8 | 84.8 |
| Lipid Content day 11 | 4 17.2 ± 0.5 \(a\) | 17.2 ± 0.7 \(a\) | 19.9 ± 0.8 \(b\) | \(64.7 \pm 2.3\) \(c\) | 62.6 ± 4.4 \(c\) | 86.7 ± 8.3 \(d\) |

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\(1\)(mean ± SEM), Biomass day 0 vs day 11: OE (\(p = 0.0005\)); PF (\(p = 0.0001\)); CO (\(p > 0.05\)); \(^2\)(mean)Estimations of body protein, water and lipid on day 0 = g/100g of CO on day 11, assuming the lack of variation in body composition over 10 days in these adult rats; \(^3\)(mean) estimation of body protein, water and lipid on day 0(g); \(^4\)(mean ± SEM) Body protein, water and lipid on day 11 (g/100g); \(^5\)(mean ± SEM) body protein, water and lipid on day 11 (g). Values sharing one superscript letter horizontally are not significantly different (\(p>0.05\)).
the rats and estimated equivalent to those of controls on day 11, assuming the lack of significant variation in this adult age. Actual variations of biomass and estimated variations of total protein, water and lipid in OE were similar to those in pair-fed group (Table 2).

Compared to PF rats, OE treatment resulted in higher plasma triacylglycerols and lower total cholesterol, while CO plasma lipid values were both significantly higher than either treated groups (Table 3). Plasma glucose and urea in OE, though lower than CO, did not differ significantly. Plasma insulin levels in CO were the highest: CO vs PF \( (p = 0.0120) \) and CO vs OE \( (p = 0.0114) \) (Table 3).

Relative weights of epididymal fat depots (g/g of total body weight x 100) were alike in the three groups, CO: 2.05 ± 0.17, OE: 2.04 ± 0.11 and PF: 2.03 ± 0.12 (ns). On the contrary, retroperitoneal fat depot relative weights were CO: 2.65 ± 0.14, OE: 2.12 ± 0.17, PF: 1.90 ± 0.10. CO was significantly higher than OE and PF \( (p = 0.02) \), while OE was not significantly higher than PF.

Though not tested yet, the activity and locomotion of the OE treated rats were evidently lower than those of controls. This unusual behaviour was transient, as the rats were apparently recovered at about day 8 of treatment. On the other hand, the activity and exploratory behaviour of pair-fed rats were indistinguishable from those of controls, without any evidence of stress derived from lower feeding.

**Discussion**

Beta strain of rats is a suitable model for human hypertriaciglycerolemia mild obesity and progressive glucose intolerance evolving towards type 2 diabetes. Food intake was significantly reduced in Beta obese rats on oleoyl-estrone treatment and of course in pair-fed counterparts. Consequently, a significant reduction in biomass compared to controls took place after 10 days on either OE or PF treatments. Body weight losses were basically from fat, accompanied by losses of water and protein in a much lower proportion. The reduction in total lipid content in OE group resulted similar to that in PF one. However, OE retroperitoneal fat depots tended to remain heavier than those in PF group, suggesting an OE specific-mediated reduction perhaps in a type of WAT depot different from those studied here. This might be mesenteric fat, since its decrease was the highest in Zucker lean rats on OE treatment. The unaltered epididymal fat depot weight suggests that the maintenance of this WAT site, associated with the species preservation, is beyond the influence of the OE dose given or the level of caloric restriction achieved in these experiments.

Plasma parameters for controls were typical for Beta mature males: hypertriaciglycerolemia, normal plasma total cholesterolemia and high glycermia baseline values.

**Table 2** – Variations of biomass and body components in 200 days-old male Beta rats with mild obesity and type 2 diabetes treated with oleoyl-estrone or pair feeding

<table>
<thead>
<tr>
<th></th>
<th>O. estrone ( (n = 6) )</th>
<th>Pair-fed ( (n = 7) )</th>
<th>Control ( (n = 5) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass(^1)</td>
<td>-51.5 ± 6.5 (^a)</td>
<td>-38.7 ± 3.3 (^a)</td>
<td>+6.4 ± 5.8 (^b)</td>
</tr>
<tr>
<td>Protein(^2)</td>
<td>-4.2</td>
<td>-3.8</td>
<td>+1.1</td>
</tr>
<tr>
<td>Water(^2)</td>
<td>-27.3</td>
<td>-18.1</td>
<td>+3.4</td>
</tr>
<tr>
<td>Lipid(^2)</td>
<td>-20.5</td>
<td>-17.1</td>
<td>+1.9</td>
</tr>
</tbody>
</table>

\(^1\) Variations of biomass (mean ± SEM); and \(^2\) estimated variations of body components (mean). Estimated variations of body protein, water and lipid = g on day 11 – g estimated on day 0. (Grams on day 0 were estimated from biomass on day 0 and % of body components of controls on day 11, assuming the lack of variation in body composition over ten days in these adult rats). Values sharing superscript letter are not significantly different \( (p>0.05) \).

**Table 3** – Plasma parameters in 200 days-old male Beta rats with mild obesity and type 2 diabetes treated with oleoyl-estrone or pair feeding

<table>
<thead>
<tr>
<th></th>
<th>Oleoyl-estrone ( (n = 10) )</th>
<th>Pair-fed ( (n = 7) )</th>
<th>Control ( (n = 8) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerols (mM)</td>
<td>2.85 ± 0.4 (^a)</td>
<td>1.54 ± 0.2 (^b)</td>
<td>4.86 ± 0.6 (^c)</td>
</tr>
<tr>
<td>Total cholesterol (mM)</td>
<td>1.21 ± 0.2 (^a)</td>
<td>2.44 ± 0.1 (^b)</td>
<td>3.02 ± 0.2 (^c)</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>11.3 ± 1.5 (^ab)</td>
<td>10.4 ± 1.4 (^a)</td>
<td>16.2 ± 2.5 (^b)</td>
</tr>
<tr>
<td>Urea (mM)</td>
<td>7.37 ± 0.6 (^ab)</td>
<td>6.19 ± 0.3 (^a)</td>
<td>8.79 ± 0.4 (^b)</td>
</tr>
<tr>
<td>Insulin (µUI/ml)</td>
<td>3.40 ± 0.5 (^a)</td>
<td>3.64 ± 0.7 (^a)</td>
<td>10.32 ± 2.4 (^b)</td>
</tr>
</tbody>
</table>

(mean ± SEM) Values sharing one superscript letter horizontally are not significantly different \( (p>0.05) \).
Both OE and PF showed lower plasma total cholesterol and triacylglycerol levels than CO. Nevertheless, these values were significantly different between OE and PF, accounting for a specific OE-mediated reduction in both types of lipids. Surprisingly, plasma triacylglycerol levels on OE treatment were less sensitive to the reduction of body weight than plasma total cholesterolemia.

Reduction in plasma triacylglycerolemia in OE treated group was lower than in PF. This could be a consequence of the increased liberation of fatty acids from the fat depots. Meanwhile, an enhanced lipoprotein turnover and lipid transport convey fatty acids to the liver, where an intense resynthesis of triacylglycerols by OE takes place. The least triacylglycerols concentration was found in PF group, suggesting a fasting-like behaviour with high fatty acid oxidation, after its nonspecific mobilization from fat depots and activation of lipoprotein lipase. Plasma total cholesterol normal values of Beta rats were practically halved on OE treatment, suggesting an improved cholesterol handling that may be, at least in part, a consequence of the OE enhancement of lipoprotein turnover. The marked reduction in plasmatic cholesterol levels indicates an alternative metabolism. As long as in these studied conditions there are no special cholesterol needs for growth or cell differentiation, the most probable cholesterol alternative metabolic sink would be the liver. For growth or cell differentiation, the most probable cholesterol alternative metabolic sink would be the liver.

Although nitrogen balance was not performed, plasma urea within the physiological range in OE group might express the lack of amino acid mobilization. The reduction in the hyperglycemic level on OE treatment failed to reach the euglycemic range in these rats. The strong reduction in insulin concentration could not be attributed to OE, but to reduced caloric intake, since it was observed with both treatments.

In conclusion, for these Beta rats OE-mediated effects resulted similar to those described by Alemany et al. in various rodent strains. Some disagreements might be attributed to differences of genetic line, age (adult stabilized Beta rats vs younger ones) and/or sex.

Although OE has been referred not to have any apparent side effects in the various rodent strains assayed, the unusual behaviour of Beta OE-treated rats would be worth pursuing in further investigations.

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References