Plasma oestrogen changes in adult male cats after orchiectomy, body-weight gain and low-dosage oestradiol administration

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Abstract

The physiological relevance of oestradiol (E2) on post-orchiectomy (OX) food intake control was evaluated in six adult, male, domestic, short-hair cats. Jugular venous plasma E2 and oestrone (E1) concentrations were determined weekly before OX and immediately after OX in a cross-over trial of two 3-week periods in which E2 (0·5 μg) or vehicle (0·1 ml/kg) was subcutaneously injected daily and blood was sampled 4 h later. Plasma E1 and E2 concentrations before OX were 32 (SE 8·3) and 4·3 (SE 1·0) pg/ml, respectively. Following OX, plasma concentrations of E2 were decreased (P=0·04) while those of E1 were unchanged. Injections of E2 increased (P=0·02) plasma E2 towards pre-OX concentrations. In a second cross-over trial, plasma E1 and E2 were determined weekly during the last 3 weeks of two 8-week periods in which food was continuously presented or restricted to 110 % of pre-OX amounts. Continuous food presentation compared with restricted food presentation resulted in greater body weight (6·4 (SE 0·4) v. 5·4 (SE 0·4) kg, P=0·02) and body fat percentage (29 (SE 3) v. 23 (SE 2) %, P=0·09) but no significant changes were observed in plasma E1 and E2 concentrations. Hence, circulating E2 appears to be reduced by OX, while it is not significantly changed by body-fat gain. The amount of E2 injected after OX was not supra-physiological; it restored plasma E2 to pre-OX concentrations and reduced food intake in five of the six cats by a mean of 14 (SE 3) %.

Key words: Orchiectomy: Body fat: Oestrone: Energy intake

After orchiectomy (OX), domestic cats gain, on average, 25–30 % in body weight, the majority of which is from accretion of body fat. The responsible physiological perturbation is not known with certainty. Kanchuk et al(1) suggested that food intakes of male and female cats are modulated by oestrogens, so that after gonadectomy, inhibition of food intake by oestrogens is diminished, as has been reported in female rodents. Indeed, the most potent of endogenous oestrogens, oestradiol (E2), circulates in the plasma of sexually intact male cats in concentrations similar to those in females except during the ovarian follicular phase(2). Oestrogen in males may be produced by the testis and extragonadally from activities of aromatases on testosterone and of other enzymes on oestrone (E1) and androstenedione(3). In later investigations, Cave et al(4,5) evinced an effect of oestrogen on food intake in cats. These investigators found that daily administration of E2 prevented post-neutering weight gain in male and female cats. They further found that E2 administration also reduced food intake in overweight gonadectomised males and females. It appeared to them that the overweight condition potentiated the food intake inhibition caused by the oestrogen.

Cave et al(4,5) reported that exogenous E2 effectively inhibited food intake at a very low dose. This dose did not appear to evoke oestrus and only modestly changed vaginal cytology from the anoestrus condition in ovarieectomised cats. These findings prompted speculation that oestrogen administration might be a useful adjunct for weight-loss treatment. With respect to this prospect, the physiological relevance of the previously described minimally effective dose of E2 for inhibiting food intake was presently investigated. Towards this aim, it was determined whether OX reduced plasma E2 concentrations in males and whether the E2 dose restored pre-OX plasma E2 concentrations in cats. Additionally, because adipose is a reputed source of oestrogen(6), it was determined whether plasma E2 and its potential circulating precursor, E1, are increased during post-OX gain in body fat mass.

Experimental methods

Animals

A total of six purpose-bred adult (1·3–1·6 years), male, domestic, short-hair cats (4·3–7·1 kg) were studied. The cats...
were housed in a controlled temperature (23–27°C) and light (12 h light–12 h dark) facility individually in lodges (1·1 × 1·6 × 2·4 m) except for 30 min daily for socialisation in a common room. Fresh water and a commercial extruded, dry-type diet, formulated for maintenance (17·9 kJ/g, Special 33; Royal Canin USA, St Charles, MO, USA) were presented daily for ad libitum consumption. Body weight and food intake were determined weekly and daily, respectively. The study was approved by the Institutional Animal Use and Care Committee of the University.

Experimental protocol

Following a pre-OX adaptation period of 3 weeks, food was withheld overnight, body fat mass was estimated and cats were neutered by the standard open technique after premedication (acetaminophen (0·02 mg/kg); atropine sulphate (0·04 mg/kg); buprenorphine (0·01 mg/kg) and anaesthesia (ketamine (10 mg/kg); diazepam (0·5 mg/kg)). Body fat mass was estimated as described previously from a dilution of subcutaneously administered sterile salinated (9 gNaCl/l) 2H2O (0·4 g/kg) in body water7.

Daily diet presentation to each cat after OX was limited to 110% of its pre-OX mean daily intake. The restriction was to prevent substantial gain in adipose and thereby theoretically limit adipose metabolism of oestrogens. For 3 weeks, beginning the day after OX, interscapular subcutaneous injections of sterile-filtered 17β-E2 (0·5 μg) dispersed in sesame oil vehicle (0·1 ml) were given daily to three cats, while injections of vehicle alone were given to the other cats. After 3 weeks, the injection treatments were crossed over and continued an additional 3 weeks (Fig. 1). Subsequently, the cats were randomly reassigned to two groups of three cats. Diet was continuously presented to one group, while to the other group, diet continued to be limited to 110% of pre-OX intake. After 8 weeks, body fat mass was estimated in all cats. Food presentation was then restricted until body weights of the cats were reduced to near pre-OX weights at rates not greater than 2% body weight/week. The diet presentation scheme was then crossed over, and 8 weeks later, body fat mass was again estimated.

During each of the last 3 weeks of each experimental period, blood (5 ml) was collected by jugular venepuncture and added to tubes containing anticoagulant (4·5 mg EDTA [K3]; Kendall Monoject, Tyco HealthCare, Mansfield, MA, USA). Plasma was extracted after centrifugation (1200 g, 10 min) and stored at –20°C until assayed for oestrogen content. During E2 and vehicle administrations, blood was sampled 4 h after injections. In a separate trial, the appropriateness of this sampling time was evaluated by determining E2 in the plasma of blood collected from each cat before and at 2, 4, 8, 16 and 24 h after a single E2 injection.

Plasma oestrogens

Plasma E1 and E2 concentrations were determined by a method developed for use on human plasma8 with the following modifications: plasma samples (1 ml) were solid-phase extracted, dried at 35°C by centrifugal evaporation, reconstituted in 2-propanol and injected (40 μl) on an HPLC column (Capcell Pak NH2 UG80 S5; Shiseido, Tokyo, Japan). E1 and E2 fractions were isocratically eluted at 1 ml/min with

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Fig. 1. Order of experimental trials, procedures and sampling. OX, orchiectomy; vh, vehicle; E2, oestradiol. *Each cat was daily presented with 110% of their pre-OX mean daily food intake.
a mobile phase, 2-propanol–hexane (3:7, v/v, HPLC grade; Fisher Scientific, Fair Lawn, NJ, USA), for which the residue left after drying did not interfere with RIA of the oestrogens. After drying by centrifugal evaporation, the oestrogens in the fractions were quantified by RIA using half the volume of reagents of commercial kits (E1 RIA, DSL-8700; Diagnostic Systems Laboratories, Webster, TX, USA and ImmunoChem™ Double Antibody, 17β-Oestradiol; ICN Biomedicals, Cosa Mesa, CA, USA, respectively). For the E1 RIA, kit standards were substituted with dried aliquots of E1 diluted in ethanol. The dried E1 standards and plasma fractions were reconstituted in 25 μl buffer, in which γ-globulin was substituted with gelatin from porcine skin (type A, Sigma; St Louis, MO, USA). For the E2 RIA, kit standards were used and plasma fractions were reconstituted with 25 μl of standard that did not contain E2. Tritiated E1 and E2 were not added to plasma samples as internal standards. The specific activities of commercially available labels were too low for use in plasma samples with low E2 concentrations. However, recoveries of radioactivity (400–800 Bq) of freshly fractionated tritiated labels (GE Healthcare, Amersham, Buckinghamshire, UK), [2,4,6,7-3H]E1 (14 GBq/mg) and [2,4,6,7,16,17-3H]E2 (20 GBq/mg), which were added to the samples of feline plasma (n 3), were high, 78 (sd 4) and 72 (sd 3) %, respectively.

Statistics

Means of daily metabolisable energy (ME) intake and weekly plasma concentrations of E1 and E2 were determined for each cat for each experimental period: pre-OX adaptation, unrestricted diet presentation, post-OX-restricted diet presentation, post-OX-restricted diet presentation. The significances of between-experimental period differences in these variables along with body weight and body fat percentage were determined using paired t tests. Linear regression analysis was used to determine the significance of correlations between variable observations within an experimental period. Statistical analyses were conducted with SAS for Windows software, version 9.1.3 (SAS Institute, Cary, NC, USA).

Results and discussion

Neutering and oestradiol injection

The mean ME intakes among the cats after OX, during the period of vehicle injections, were not significantly different from those observed during the pre-OX period (Table 1). An increase in ME intake after OX was expected(19). The cause for the lacking change in food intake is unknown. The post-OX limiting of diet amount to 110 % of the pre-OX ad libitum intake is probably contributing. In order to control for temporal variation in environmental factors that might affect food intake, studies showing that OX increases food intake in male cats compare simultaneous observations between OX and sexually intact controls(11). During the post-OX period, food intakes among all cats during injections of E2 were not significantly different compared with those during vehicle injections (Table 1). However, in five of the six cats, food intakes during E2 injections relative to vehicle injections were less, a mean of 14 (sd 3) % less. For reference, OX of cats results in mean increases of food intake of 12–15 % (1). Body weights of cats not responding to E2 injections (7·1 kg) were substantially greater than those of the other cats (4·3–5·3 kg); hence, the E2 dosage for cats was the lowest used (0·07 v. 0·9–0·12 μg/kg).

Plasma E2 concentrations during the post-OX period when vehicle injections were given were lower (P = 0·04) than the concentrations before OX (Table 1). When E2 injections were given during the post-OX period, plasma E2 concentrations at 4 h after injections were greater (P = 0·02) relative to those when the vehicle injections were given and not significantly different from pre-OX plasma E2 concentrations. The means of plasma E2 concentrations after a single E2 injection were 190, 149, 124, 133 and 160 % of baseline concentrations at 2, 4, 8, 16 and 24 h, respectively. The time-weighted arithmetic mean of plasma E2 concentrations over the 24 h period was 147 % of baseline. Hence, the plasma E2 concentrations at the 4 h sampling time appeared to be representative of the elevation of E2 in plasma following E2 injections.

Plasma E1 concentrations before OX were not significantly different after OX or E2 administration (Table 1).

Table 1. Effects of orchiectomy (OX), oestrogen replacement and ad libitum consumption of diet on study outcomes in adult male cats (Mean values with their standard errors, n 6)

<table>
<thead>
<tr>
<th>Condition/treatment</th>
<th>Pre-OX</th>
<th>Vehicle</th>
<th>E2</th>
<th>Restricted</th>
<th>Ad libitum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>5·2</td>
<td>0·3</td>
<td>5·3</td>
<td>0·4</td>
<td>5·4</td>
</tr>
<tr>
<td>Body fat mass (%)</td>
<td>21·9</td>
<td>0·5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Food intake (kJ/kg per d)</td>
<td>314</td>
<td>10</td>
<td>292</td>
<td>17</td>
<td>264</td>
</tr>
<tr>
<td>Plasma E2 (pg/ml)</td>
<td>4·3</td>
<td>1·0</td>
<td>2·1†</td>
<td>0·3</td>
<td>3·8‡</td>
</tr>
<tr>
<td>Plasma E1 (pg/ml)</td>
<td>32·0</td>
<td>8·3</td>
<td>27·6</td>
<td>8·0</td>
<td>27·8</td>
</tr>
</tbody>
</table>

E2, estradiol; ND, not determined; E1, estrone.
* Mean value was greater than the restricted values (P = 0·01).
† Tended to be greater than the restricted values (P = 0·09).
‡ Less than the pre-OX values (P = 0·04).
§ Tended to be greater than the vehicle value (P = 0·02).

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These concentrations were approximately five- to ten-fold greater than E₂ concentrations, a condition observed in other species\(^{(8)}\).

**Post-neutering weight gain**

The mean daily ME intake during continuous presentation of the diet for 8 weeks was substantially greater \((P=0.01)\) than that when diet presentation was restricted to 110\% of pre-OX intake (Table 1). At the end of the period of continuous diet presentation, great variation among the cats was observed in body weights (8.2–5.0 kg) and fat percentages (19–32\%). Nonetheless, the body weights were greater \((P=0.01)\) and body fat percentages tended to be greater \((P=0.09)\) than those at the end of the periods of restricted diet access. Additionally, the body fat percentages tended to be positively correlated \((r=0.78, P=0.07)\) with mean daily ME intakes during the periods of unrestricted diet presentation.

While food intake and adiposity appeared affected by the degree of diet presentation, the abundance of circulating oestrogens appeared unaffected. The E₁ and E₂ concentrations in plasma were not significantly different between the restricted and unrestricted periods of diet presentation (Table 1). They were also not significantly correlated with body fat percentage at the end of the periods of unrestricted diet presentation. Hence, expansion of adipose mass following OX does not appear to substantially affect circulating concentrations of E₁ and E₂ in cats.

In conclusion, E₁ and a portion of E₂ in the plasma of male cats appear to originate from extragonadal sources. The plasma E₂ concentrations appear to be reduced by about half following OX. Exogenous E₂ at a dose reported to reduce food intake appears to restore pre-OX plasma E₂ concentrations.

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**References**