British Journal of Nutrition (2011), **106**, S15–S18
doi:10.1017/S0007114511001796

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

Robert Backus*

Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, A384 Clydesdale Hall, 900 East Campus Drive, Columbia, MO 65211, USA

(Received 15 October 2010 – Revised 23 March 2011 – Accepted 7 March 2011)

Abstract

The physiological relevance of oestradiol (E_2) on post-orchiectomy (OX) food intake control was evaluated in six adult, male, domestic, short-hair cats. Jugular venous plasma E_2 and oestrone (E_1) concentrations were determined weekly before OX and immediately after OX in a cross-over trial of two 3-week periods in which E_2 (0·5 μg) or vehicle (0·1 ml/kg) was subcutaneously injected daily and blood was sampled 4 h later. Plasma E_1 and E_2 concentrations before OX were 32 (SE 8·3) and 4·3 (SE 1·0) pg/ml, respectively. Following OX, plasma concentrations of E_2 were decreased ($P=0·04$) while those of E1 were unchanged. Injections of E_2 increased ($P=0·02$) plasma E_2 towards pre-OX concentrations. In a second cross-over trial, plasma E_1 and E_2 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 E_1 and E_2 concentrations. Hence, circulating E_2 appears to be reduced by OX, while it is not significantly changed by body-fat gain. The amount of E_2 injected after OX was not supra-physiological; it restored plasma E_2 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 (E_2), 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 (E_1) 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 E_2 prevented post-neutering weight gain in male and female cats. They further found that E_2 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 E_2 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 ovariectomised 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 E_2 for inhibiting food intake was presently investigated. Towards this aim, it was determined whether OX reduced plasma E_2 concentrations in males and whether the E_2 dose restored pre-OX plasma E_2 concentrations in cats. Additionally, because adipose is a reputed source of oestrogen[6], it was determined whether plasma E_2 and its potential circulating precursor, E_1, 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

Abbreviations: E_1, oestrone; E_2, oestradiol; ME, metabolisable energy; OX, orchiectomy.

Presented as an oral abstract at the WALTHAM International Nutritional Sciences Symposium, Cambridge, UK, 16 September 2010.
* **Corresponding author:** Associate Professor R. Backus, fax +1 573 884 5444, email backusr@missouri.edu

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 pre-medication (acetylpromazine (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) ^2H_2O (0·4 g/kg) in body water[7].

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-E_2 (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 [K_3]; 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 E_2 and vehicle administrations, blood was sampled 4 h after injections. In a separate trial, the appropriateness of this sampling time was evaluated by determining E_2 in the plasma of blood collected from each cat before and at 2, 4, 8, 16 and 24 h after a single E_2 injection.

Plasma oestrogens

Plasma E_1 and E_2 concentrations were determined by a method developed for use on human plasma[8] 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). E_1 and E_2 fractions were isocratically eluted at 1 ml/min with

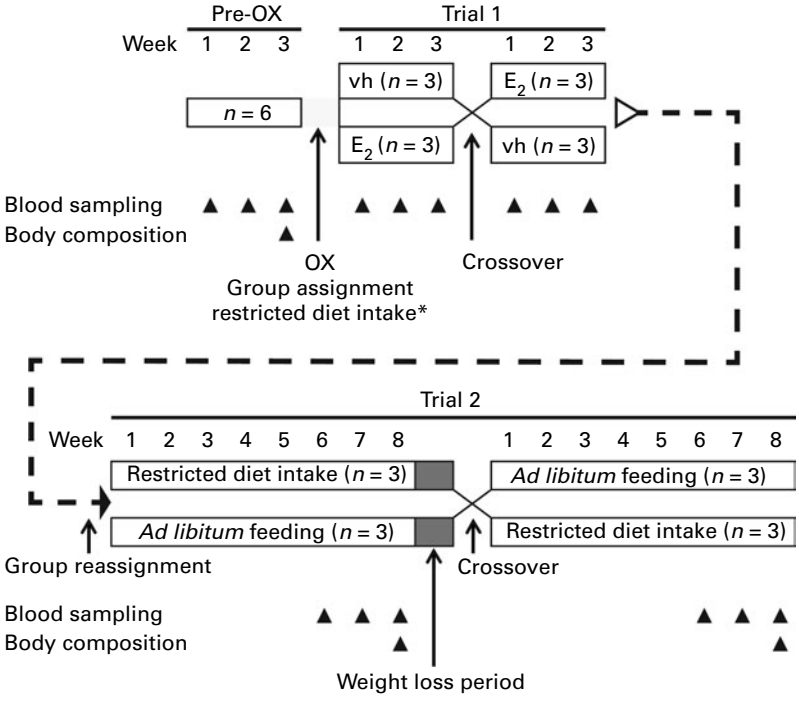

Fig. 1. Order of experimental trials, procedures and sampling. OX, orchiectomy; vh, vehicle; E_2, oestradiol. *Each cat was daily presented with 110 % of their pre-OX mean daily food intake.

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 (E_1 RIA, DSL-8700; Diagnostic Systems Laboratories, Webster, TX, USA and ImmunoChem™ Double Antibody, 17β-Oestradiol; ICN Biomedicals, Cosa Mesa, CA, USA, respectively). For the E_1 RIA, kit standards were substituted with dried aliquots of E_1 diluted in ethanol. The dried E_1 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 E_2 RIA, kit standards were used and plasma fractions were reconstituted with 25 μl of standard that did not contain E_2. Tritiated E_1 and E_2 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 E_2 concentrations. However, recoveries of radioactivity (400–800 Bq) of freshly fractionated tritiated labels (GE Healthcare, Amersham, Buckinghamshire, UK), [2,4,6,7-^{3}H]E_1 (14 GBq/mg) and [2,4,6,7,16,17-^{3}H]E_2 (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 E_1 and E_2 were determined for each cat for each experimental period: pre-OX adaptation, post-OX E_2 injection, post-OX vehicle injection, post-OX unrestricted 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[9]. 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[1].

During the post-OX period, food intakes among all cats during injections of E_2 were not significantly different compared with those during vehicle injections (Table 1). However, in five of the six cats, food intakes during E_2 injections relative to vehicle injections were less, a mean of 14 (SE 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 E_2 injections (7·1 kg) were substantially greater than those of the other cats (4·3–5·3 kg); hence, the E_2 dosage for cats was the lowest used (0·07 *v.* 0·9–0·12 μg/kg).

Plasma E_2 concentrations during the post-OX period when vehicle injections were given were lower (P=0·04) than the concentrations before OX (Table 1). When E_2 injections were given during the post-OX period, plasma E_2 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 E_2 concentrations. The means of plasma E_2 concentrations after a single E_2 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 E_2 concentrations over the 24 h period was 147% of baseline. Hence, the plasma E_2 concentrations at the 4 h sampling time appeared to be representative of the elevation of E_2 in plasma following E_2 injections.

Plasma E_1 concentrations before OX were not significantly different after OX or E_2 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)

			Trial 1				Trial 2			
	Pre-OX		Vehicle		E_2		Restricted		*Ad libitum*	
Condition/treatment	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body weight (kg)	5·2	0·3	5·3	0·4	5·4	0·3	5·7	1·0	6·1*	0·4
Body fat mass (%)	21·9	0·5	ND	ND	ND	ND	22·8	3·2	28·7†	2·0
Food intake (kJ/kg per d)	314	10	292	17	264	9	218	13	261*	17
Plasma E_2 (pg/ml)	4·3	1·0	2·1‡	0·3	3·8§	0·5	2·1	0·5	2·9	0·7
Plasma E_1 (pg/ml)	32·0	8·3	27·6	8·0	27·8	8·1	21·2	7·2	22·6	6·9

E_2, estradiol; ND, not determined; E_1, 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).

British Journal of Nutrition

These concentrations were approximately five- to ten-fold greater than E_2 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 ($P=0{\cdot}01$) 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 ($P=0{\cdot}01$) and body fat percentages tended to be greater ($P=0{\cdot}09$) 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, $P=0{\cdot}07$) 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 E_1 and E_2 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 E_1 and E_2 in cats.

In conclusion, E_1 and a portion of E_2 in the plasma of male cats appear to originate from extragonadal sources. The plasma E_2 concentrations appear to be reduced by about half following OX. Exogenous E_2 at a dose reported to reduce food intake appears to restore pre-OX plasma E_2 concentrations.

Acknowledgements

There are no conflicts of interests known to the author. The present study received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

1. Kanchuk ML, Backus RC, Calvert CC, *et al.* (2003) Weight gain in gonadectomized normal and lipoprotein lipase-deficient male domestic cats results from increased food intake and not decreased energy expenditure. *J Nutr* **133**, 1866–1874.
2. Perez JF, Conley AJ, Dieter JA, *et al.* (1999) Studies on the origin of ovarian interstitial tissue and the incidence of endometrial hyperplasia in domestic and feral cats. *Gen Comp Endocrinol* **116**, 10–20.
3. Vermeulen A, Kaufman JM, Goemaere S, *et al.* (2002) Estradiol in elderly men. *Aging Male* **5**, 98–102.
4. Cave NJ, Backus RC, Marks SL, *et al.* (2007) Oestradiol and genistein reduce food intake in male and female overweight cats after gonadectomy. *N Z Vet J* **55**, 113–119.
5. Cave NJ, Backus RC, Marks SL, *et al.* (2007) Oestradiol, but not genistein, inhibits the rise in food intake following gonadectomy in cats, but genistein is associated with an increase in lean body mass. *J Anim Physiol Anim Nutr (Berl)* **91**, 400–410.
6. Wake DJ, Strand M, Rask E, *et al.* (2007) Intra-adipose sex steroid metabolism and body fat distribution in idiopathic human obesity. *Clin Endocrinol (Oxf)* **66**, 440–446.
7. Backus RC, Cave NJ, Ganjam VK, *et al.* (2010) Age and body weight effects on glucose and insulin tolerance in colony cats maintained since weaning on high dietary carbohydrate. *J Anim Physiol Anim Nutr (Berl)* **94**, e318–e328.
8. Yasui T, Yamada M, Kinoshita H, *et al.* (1999) Combination of automatic HPLC-RIA method for determination of estrone and estradiol in serum. *J Clin Lab Anal* **13**, 266–272.
9. Backus RC, Cave NJ & Keisler DH (2007) Gonadectomy and high dietary fat but not high dietary carbohydrate induce gains in body weight and fat of domestic cats. *Br J Nutr* **98**, 641–650.

British Journal of Nutrition