Effects of estrogen on thermoregulatory evaporation in rats exposed to heat

M. A. BAKER, D. D. DAWSON, C. E. PETERS, AND A. M. WALKER
Division of Biomedical Sciences, University of California, Riverside, California 92521

Baker, M. A., D. D. Dawson, C. E. Peters, and A. M. Walker. Effects of estrogen on thermoregulatory evaporation in rats exposed to heat. *Am. J. Physiol.* 267 (*Regulatory Integrative Comp. Physiol.* 36): R673–R677, 1994.—The purpose of this study was to determine the effects of estrogen (E_2) replacement on thermoregulation in ovariectomized rats exposed to heat. Female Sprague-Dawley rats were ovariectomized and splenectomized and implanted with a temperature-sensitive transmitter. Each rat was studied when E_2 treated (after an E_2 pellet implant) and untreated. Animals were divided into two groups with opposite order of treatment and were studied over a 9-wk period. Measurements of body core temperature (T_c) and evaporative water loss (EWL) were made on unrestrained animals resting at 38°C air temperature. E_2-treated animals increased EWL at all levels of T_c, reduced the threshold T_c for onset of saliva spreading, and regulated T_c at a lower level during heat exposure. E_2 treatment elevated plasma E_2 and reduced hematocrit but did not affect plasma osmolality. These effects of E_2 on evaporative cooling and T_c in heat-stressed rats are similar to those that have been reported in human females. The mechanisms of the thermoregulatory effects of E_2 remain to be studied.

heat stress; rat thermoregulation; estrogen replacement

ESTROGEN REPLACEMENT THERAPY is prescribed extensively for postmenopausal women because it reduces the risk of osteoporosis and of cardiovascular disease (2, 12). Another possibly advantageous effect of estrogen was reported recently by Tankersley et al. (27) who found that in middle-aged women exercising in a warm environment, estrogen replacement lowered threshold body temperature for onset of sweating and cutaneous vasodilatation and reduced body core temperature. The mechanisms of these effects of estrogen during heat stress are not known and could involve direct or indirect actions on peripheral effectors or on central nervous thermoregulatory control regions or could be secondary to other systemic effects such as alterations in body fluid balance.

We asked whether estrogen has similar effects on thermoregulation in nonhuman species, and whether the rat, an animal that uses saliva spreading for evaporative cooling (26), might be a useful animal for studying the mechanisms of action of estrogen on thermoregulation in heat stress. In this study we have measured the effects of sustained estrogen replacement on thermoregulatory evaporation and body core temperature in ovariectomized rats resting in a hot environment.

METHODS

Animals and surgical procedures. Thirteen female Sprague-Dawley rats weighing 272 ± 3 g at the time of surgery were used. They were housed in group cages in a temperature-controlled animal facility (20–25°C) with a 12:12-h light-dark cycle and were transported to the laboratory for experiments. To accustom them to the conditions of the experiment, for 8 wk they were handled daily and placed into the warm recording chamber 3–4 times/wk. At the end of this period, each animal was ovariectomized and splenectomized and a temperature-sensitive AM radio transmitter weighing 2.3 g (MiniMitter, Sunriver, OR) was implanted into the peritoneal cavity. The spleen was removed so that changes in plasma volume could be estimated from hematocrit (Hct) changes without the possibility of splenic sequestration and release of erythrocytes. Surgery was performed under pentobarbital sodium anesthesia (40 mg/kg ip) using sterile technique.

Experimental protocol. The experiment was designed so that the same animals could be studied when estrogen (E_2) treated and untreated. Animals were divided into two groups with opposite order of exposure to E_2. *Group 1* ($n = 6$) received an E_2 implant in *week 0* of the study (10 days after the initial surgery) and *group 2* ($n = 7$) remained without E_2 until *week 3* (28 days after surgery, Table 1). Estrogen administration was continued in *group 2* when we found, unexpectedly, that the first E_2 implant at *week 3* did not affect Hct or thermoregulatory evaporation. The second implant was given in *week 6*.

Estrogen pellets [0.25 mg 17β-estradiol, Innovative Research of America (IRA), Toledo, OH] were implanted subcutaneously on the back of the neck through a 2-mm skin incision while the animal was anesthetized briefly with Metofane. According to IRA, these pellets will release E_2 at a constant rate for 3 wk.

Measurements of thermoregulatory responses were made between 1000 and 1400 h on *days 1–4* of *weeks 2*, *5*, and *8*. On *day 5* of *weeks 0*, *1*, *2*, *3*, *4*, *5*, and *8*, blood samples were taken from the tip of the tail for measurement of Hct, plasma osmolality (p_{Osm}), and plasma E_2 (Table 1). Sample size was ~125 μl when only Hct was to be measured and ~1 ml when P_{Osm} and E_2 were measured as well.

For measurements of body core temperature (T_c) and evaporative water loss (EWL) during heat exposure, each rat was brought to the laboratory and placed in a clear Lucite chamber (23 cm long × 18 cm wide × 23 cm high) within an environmental room with an observation window. The animal could move about freely on a wire mesh platform over mineral oil, which prevented evaporation from urine and feces. Temperature of air in the animal chamber (T_a) was maintained at 38 ± 0.2°C. Measurements of EWL and T_c were started 3–4 min after the rat was placed in the chamber and continued for 90 min.

Techniques of measurement. Evaporative water loss was measured continuously as described previously (28). The chamber was ventilated with compressed air flowing at 6 l/min, and the relative humidity and temperature of air entering and leaving the chamber were monitored with Vaisala probes (Vaisala Oy, Helsinki, Finland). Rates of EWL were calculated every 10 s and averaged over 5-min intervals by a computerized data-collection system (22). Air temperature in the animal chamber was measured every 30 s using a copper-constantan thermocouple and the same computerized system. T_c was measured every 5 min by counting the pulse rate of the implanted transmitter using a radio receiver near the cage and an earphone outside of the chamber.

Table 1. *Effects of estrogen implants on hematocrit, plasma estrogen and osmolality, and body weight in ovariectomized rats during a 9-wk study*

	n	*Week 0*	*Week 1*	*Week 2*	*Week 3*	*Week 4*	*Week 5*	*Week 8*
Group 1	6							
Hct, %		43.8 ± 0.5	44.4 ± 0.4^{NS}	41.6 ± 0.6†	41.3 ± 0.4‡	44.0 ± 0.5	44.2 ± 0.6	44.0 ± 0.4
E_2, pg/ml				46.1 ± 5.7‡			25.2 ± 5.5	13.3 ± 5.2
P_{Osm}, mosmol/kgH_2O				291 ± 3^{NS}				294 ± 2
Body wt, g		265 ± 4	264 ± 2‡	265 ± 4‡	269 ± 4‡	273 ± 3	277 ± 4	279 ± 4
Group 2	7							
Hct, %		44.6 ± 0.8	45.2 ± 1.0	45.2 ± 0.7	45.5 ± 0.6	45.9 ± 0.8^{NS}	43.4 ± 0.7^{NS}	41.6 ± 0.5†
E_2, pg/ml				< 1.4			65.5 ± 5.1‡	77.9 ± 4.9‡
P_{Osm}, mosmol/kgH_2O				296 ± 2				290 ± 2^{NS}
Body wt, g		277 ± 5	296 ± 5	319 ± 7	322 ± 7	294 ± 5†	296 ± 6*	284 ± 6^{NS}

Values are means ± SE. E_2, estrogen; Hct, hematocrit; P_{Osm}, plasma osmolality. *Week 0* measurements are 10 days after ovariectomy. E_2 pellets implanted at *Week 0* (*group 1*) and at *Week 3* and *Week 6* (*group 2*). *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$: significantly different from untreated animals. NS, not significantly different from untreated animals.

Hematocrit was measured in triplicate immediately after bleeding using microhematocrit tubes and centrifuge. For measurement of plasma osmolality and E_2, whole blood was centrifuged immediately after collection and aliquots of plasma were stored at −70°C. Plasma osmolality was measured with a Wescor 1500B vapor pressure osmometer. Plasma E_2 levels were measured by radioimmunoassay using a Diagnostic Products kit (Los Angeles, CA) with some modifications of the procedure. E_2 was extracted from 200 μl plasma with 2 ml ether, dried under nitrogen, and concentrated twofold by subsequent dissolution in 100 μl assay buffer. To correct for losses during storage and extraction, plasma from male or ovariectomized female rats was spiked with standard concentrations of E_2 and taken through the same procedures to serve as the standard curve. Preliminary measurements showed that this curve was parallel to the one generated using the standards in the kit. All samples were placed in the same assay and were coextracted with standards. According to the manufacturer, the detection limit for this assay is ~1.4 pg/ml and intra-assay variation is < 10%. According to Lye et al. (17) the primary antiserum exhibits cross-reactivity of 6% with 17β-estradiol-3β-D-glucuronide, 1.3% with estrone, and 0.235% with estriol, with minimal or no cross-reactivity to other naturally occurring steroids.

Statistics. Effects of E_2 treatment and of heat exposure were evaluated with paired and unpaired *t* tests, two-way analysis of variance (ANOVA) with replication, and linear regression analysis (Abacus Concepts StatView and Super ANOVA statistical software packages). Piecewise linear regression techniques (20) were used to estimate the threshold T_c for initiation of thermoregulatory evaporation in each experiment, and the effect of E_2 treatment on these thresholds was evaluated by ANOVA.

RESULTS

By *week 2*, the animals in *group 1* had higher plasma E_2 levels and lower Hct than *group 2*, but P_{Osm} was not different in the two groups (Table 1). EWL during heat exposure in *group 1* was significantly higher than in the untreated animals ($P < 0.001$ by ANOVA). In *group 1*, Hct returned to preimplant levels by *week 4*. Plasma E_2 fell between *weeks 2* and *5* and continued to fall until *week 8*. Plasma osmolality was not different between *week 2* and *week 8*. Body weights of the animals in *group 1* remained steady until *week 4* and rose slightly from *weeks 4* to *8*.

In *group 2*, which did not receive an estrogen pellet until 28 days after ovariectomy (*week 3*), the effects of E_2 administration on Hct and EWL took longer to manifest themselves (Table 1). Plasma E_2 levels were elevated, but Hct and EWL during heat exposure were not changed by *week 5* and so an additional E_2 pellet was implanted at *week 6*. By *week 8*, Hct was reduced and EWL was elevated both above the levels measured before E_2 treatment in this group ($P < 0.01$) and above the levels measured in *group 1* in *week 8* ($P < 0.01$). Plasma osmolality did not change between *week 2* and *week 8* and was not different from P_{Osm} of untreated animals in *week 8*. Body weights of the animals in *group 2* rose steadily until *week 3* and then began to drop.

Data from measurements made during *week 2* and *week 8* were pooled for final analysis of E_2 effects on the 13 animals, after confirming that there was no difference in EWL in the two groups when they were untreated (*week 8* for *group 1*, *week 2* for *group 2*) and no difference when they were treated with E_2 (*week 2* for *group 1* and *week 8* for *group 2*). Estrogen treatment had significant effects on Hct and on plasma E_2 (Table 2). There was a tendency for P_{Osm} to be lower in E_2-treated animals, but this was not significant ($P = 0.055$). Body weight was lower in E_2-treated animals, an effect of estrogen that is commonly observed in ovariectomized rats (15).

Estrogen-treated animals had significantly higher rates of EWL during heat exposure than untreated animals (Fig. 1*A*, $P < 0.001$). The pattern of change in EWL in response to heat was similar in untreated and E_2-treated animals, with a period of steady or declining EWL for the first 20–30 min of heat exposure, followed by a steep rise and a tendency to stabilize at ~60 min.

Table 2. *Effect of estrogen treatment on hematocrit, plasma estrogen and osmolality, and body weight of ovariectomized rats*

	Hct, %	E_2, pg/ml	P_{Osm}, mosmol/kgH_2O	Body wt, g
Condition				
E_2-treated	41.6 ± 0.4‡	62 ± 21‡	290 ± 2^{NS}	275 ± 5‡
Untreated	44.6 ± 0.5	6 ± 3	295 ± 1	300 ± 7

Values are means ± SE; $n = 13$ rats (data from *weeks 2* and *8*; see Table 1). ‡$P < 0.001$: Significantly different from untreated condition by paired *t* test. NS, not significantly different from untreated condition.

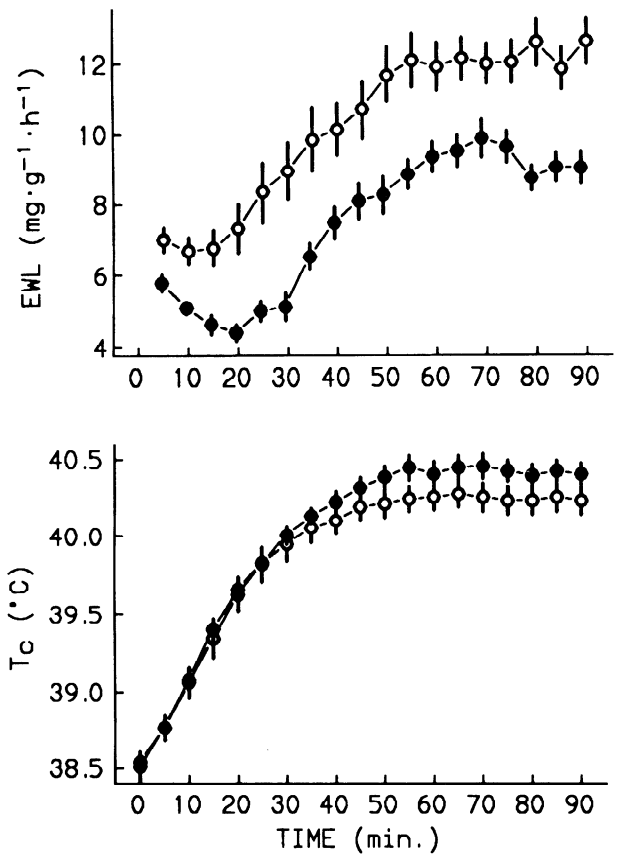

Fig. 1. Evaporative water loss (EWL) and body core temperature (T_c) of ovariectomized rats (n = 13) resting at 38°C air temperature when treated with estrogen (E_2) (○) and when untreated (●).

Observation of the animals confirmed that the beginning of the rise in the rate of evaporation coincided with the onset of saliva spreading.

Body core temperature was the same in E_2-treated and untreated animals at the beginning of the measurement period, increased markedly during the first 30–50 min of heat exposure, and remained steady until the end of the experiment (Fig. 1*B*). T_c stabilized at a lower level in E_2-treated animals, and there was a significant effect of estrogen treatment on T_c during the 90 min of heat exposure ($P < 0.001$).

The rate of evaporation at a given T_c was higher for E_2-treated animals during the entire period of measurement, and the threshold T_c at which EWL began to rise was lower (Fig. 2). Analysis of the piecewise linear regression of EWL on T_c for the first 60 min of each experiment was used to determine the point at which the slope of the line changed, and this was defined as the threshold T_c for saliva spreading. Core temperature threshold was 39.57 ± 0.07°C for E_2-treated rats and 39.82 ± 0.07°C for untreated animals ($P < 0.05$). E_2 had no effect on the slopes of the lines relating EWL to T_c.

DISCUSSION

In these experiments, maintenance of plasma estrogen at levels observed in intact rats during late diestrus and proestrus (13) had a marked effect on thermoregulation in ovariectomized rats resting at high T_a. Estrogen-treated animals had higher rates of EWL for any given T_c, began spreading saliva at a lower T_c, and regulated T_c at a lower level in the heat than untreated animals. These effects of estrogen on body temperature and on evaporative cooling in rats are remarkably similar to those observed in human females during exercise in a warm environment (27).

The similarity of the influence of estrogen on thermoregulatory evaporation in two species with such different modes of evaporative heat loss suggests that the effect could be mediated through central neural regions controlling body temperature. The preoptic area of the brain plays a major role in mammalian thermoregulation because of the presence of thermosensitive neurons that can influence thermoregulatory effectors (4). The preoptic area also contains neurons that bind estrogen (23), and estrogen can affect the firing rates of thermosensitive neurons in preoptic slice preparations (24). However, Marrone (19) found no effect of preoptic E_2 implants on T_c of rats at 24°C T_a. Studies of thermoregulation after preoptic estrogen administration in animals exposed to heat will be necessary to test the possibility that the effects we have observed are mediated by a direct action of estrogen on the preoptic area.

The influence of estrogen on thermoregulation in heat stress could also be related to estrogen-induced changes in the osmolality or volume of body fluids. In a number of mammalian species, the rate of thermoregulatory evaporation in warm environments is reduced when plasma or cerebrospinal fluid osmolality is elevated by dehydration or by administration of hypertonic fluids (1, 14, 26) and evaporative water loss is increased when hypotonic fluids are administered (8, 21, 29). Reduced P_{Osm} was reported by Skowsky et al. (25) in rats treated with estrogen but was not observed by Barron et al. (3). Although P_{Osm} in the present study tended to be lower in E_2-treated animals, the difference in P_{Osm} between treated and untreated animals was not significant, and it is unlikely that the elevation in EWL was stimulated by hyposmolality.

An expansion of plasma volume accompanies estrogen administration in both rats and humans (3, 9, 16, 27). The drop in Hct in our E_2-treated animals is a strong

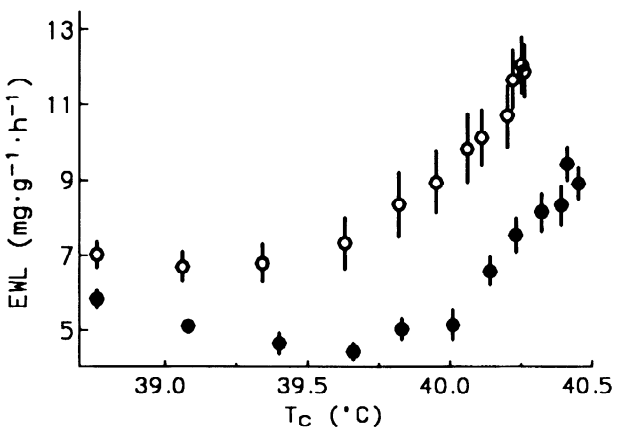

Fig. 2. Relationship of EWL to T_c in ovariectomized rats (n = 13) resting at 38°C air temperature when E_2-treated (○) and untreated (●). Data from the first 60 min of heat exposure are plotted.

indication of plasma volume expansion, since Barron et al. (3) showed that E_2 treatment in rats increases plasma volume and reduces Hct with no change in erythrocyte volume. A reduction in plasma volume below normal levels in male rats exposed to hot environments reduces the rate of salivation and elevates body temperature (14, 26), but the effects of plasma volume expansion on evaporative cooling in rodents have not been studied. In exercising human males, acute hypovolemia reduces sweat rate below control levels, but hypervolemia has no effect on sweat rate or T_c (10). In the present experiments, significant increases in EWL in E_2-treated rats were associated with decreased Hct within 2 wk of E_2 treatment in *group 1* and not until 5 wk of E_2 treatment in *group 2*. Plasma volume expansion may play a role in the thermoregulatory changes that we have observed. On the other hand, the coincident increases in EWL and in plasma volume could both depend on the activation of estrogen receptors in the brain or in other tissues.

A difference in responsiveness to estrogen after a delay in replacement such as we observed in the animals in *group 2* has been noted in other studies of estrogen effects on ovariectomized rats (15). These findings are most likely related to the influence of endogenous and exogenous steroids on the level of steroid receptors in target tissues (7).

The present experiments show an effect of estrogen on the level at which T_c is regulated in heat stress but do not help to resolve the question of whether estrogen affects body temperature of rats in thermoneutral environments. Laudenslager et al. (15) found that maintenance of plasma E_2 at levels similar to those in the E_2-treated animals in the present study did not affect T_c of ovariectomized rats at T_a of 2.5, 10, 20, and 30°C. However, Fregly et al. (11) found that E_2 treatment reduced T_c in restrained rats at T_a of 25–27°C, and Marrone et al. (19) reported an elevation in T_c after E_2 treatment in ovarectomized rats at 24°C T_a, but plasma E_2 was not measured in either study. The conflicting findings from these different studies could relate to differences in E_2 levels in the animals or to differences in the conditions of the experiment and the methods of measuring T_c.

If the enhancement of evaporative cooling produced by estrogen has any beneficial effect for normal animals, it may be during pregnancy. Pregnant rats (30) and humans (6) show adjustments in thermoregulation during heat stress that are similar to those associated with estrogen replacement, namely a reduced threshold temperature for initiating evaporative cooling and a reduction in the level at which T_c is regulated. This readjustment in maternal thermoregulation is clearly advantageous, since fetal growth and development are adversely affected by hyperthermia (18). The level of circulating estrogen is increased in most mammals during pregnancy (5) and may underlie the maternal thermoregulatory adjustments that protect the heat-sensitive fetus.

Address for reprint requests: M. A. Baker, Division of Biomedical Sciences, Univ. of California, Riverside, CA 92521.

Received 7 January 1994; accepted in final form 15 March 1994.

REFERENCES

1. **Baker, M. A.** Thermoregulation in dehydrated vertebrates. *Prog. Biometeorol.* 7: 101–107, 1989.
2. **Barrett-Connor, E.** Risks and benefits of replacement estrogen. *Annu. Rev. Med.* 43: 239–251, 1992.
3. **Barron, W. M., J. Schreiber, and M. D. Lindheimer.** Effect of ovarian sex steroids on osmoregulation and vasopressin secretion in the rat. *Am. J. Physiol.* 250 (*Endocrinol. Metab.* 13): E352–E361, 1986.
4. **Bligh, J.** Temperature regulation in mammals and other vertebrates. In: *Frontiers of Biology*. New York: Elsevier, 1973, vol. 30, p. 18–23.
5. **Catchpole, H. R.** Hormonal mechanisms in pregnancy and parturition. In: *Reproduction in Domestic Animals* (4th ed.), edited by P. T. Cupps. New York: Academic, 1991, p. 361–383.
6. **Clapp, J. F., III.** The changing thermal response to endurance exercise during pregnancy. *Am. J. Obstet. Gynecol.* 165: 1684–1689, 1991.
7. **Clark, J. H., and B. M. Markaverich.** Actions of ovarian steroid hormones. In: *The Physiology of Reproduction*, edited by E. Knobil and J. D. Neill. New York: Raven, 1988, vol. I, p. 675–724.
8. **Doris, P. A.** Osmotic regulation of evaporative water loss and body temperature by intracranial receptors in the heat-stressed cat. *Pfluegers Arch.* 398: 337–340, 1983.
9. **Fortney, S. M., W. S. Beckett, A. J. Carpenter, J. Davis, H. Drew, N. D. LaFrance, J. A. Rock, C. G. Tankersley, and N. B. Vroman.** Changes in plasma volume during bed rest: effects of menstrual cycle and estrogen administration. *J. Appl. Physiol.* 65: 525–533, 1988.
10. **Fortney, S. M., E. R. Nadel, C. B. Wenger, and J. R. Bove.** Effect of blood volume on sweating rate and body fluids in exercising humans. *J. Appl. Physiol.* 51: 1594–1600, 1981.
11. **Fregly, M. J., D. L. Kelleher, and D. J. Black.** Tolerance of estrogen-treated rats to acute cold exposure. *J. Appl. Physiol.* 47: 59–66, 1979.
12. **Harlap, S.** The benefits and risks of hormone replacement therapy: an epidemiologic overview. *Am. J. Obstet. Gynecol.* 166: 1986–1992, 1992.
13. **Henderson, S. R., C. Baker, and G. Fink.** Oestradiol-17β and pituitary responsiveness to luteinizing hormone releasing factor in the rat: a study using rectangular pulses of oestradiol-17β monitored by non-chromatographic radioimmunoassay. *J. Endocrinol.* 73: 441–453, 1977.
14. **Horowitz, M., and U. Meiri.** Thermoregulatory activity in the rat: effects of hypohydration, hypovolemia and hypertonicity and their interaction with short-term heat acclimation. *Comp. Biochem. Physiol. A Comp. Physiol.* 82: 577–582, 1985.
15. **Laudenslager, M. L., C. W. Wilkinson, H. J. Carlisle, and H. T. Hammel.** Energy balance in ovariectomized rats with and without estrogen replacement. *Am. J. Physiol.* 238 (*Regulatory Integrative Comp. Physiol.* 7): R400–R405, 1980.
16. **Lehtovirta, P.** Haemodynamic effects of combined oestrogen/progestogen oral contraceptives. *J. Obstet. Gynaecol. Br. Commonw.* 81: 517–525, 1974.
17. **Lye, S. J., B. J. Nicholson, M. Mascarenhas, L. MacKenzie, and T. Petrocelli.** Increased expression of connexin-43 in the rat myometrium during labor is associated with an increase in the plasma estrogen:progesterone ratio. *Endocrinology* 132: 2380–2386, 1993.
18. **McMurray, R. G., and V. L. Katz.** Thermoregulation in pregnancy. Implications for exercise. *Sports Med.* 10: 146–158, 1990.
19. **Marrone, B. L., R. T. Gentry, and G. N. Wade.** Gonadal hormones and body temperature in rats: effects of estrous cycles, castration and steroid replacement. *Physiol. Behav.* 17: 419–425, 1976.
20. **Neter, J., and W. Wasserman.** *Applied Linear Statistical Models*. Homewood, IL: Irwin, 1974, p. 313–315.
21. **Nielsen, B.** Effects of changes in plasma volume and osmolarity on thermoregulation during exercise. *Acta Physiol. Scand.* 90: 725–730, 1974.

22. **Nijland, M. J. M., and M. A. Baker.** Effects of hydration state on exercise thermoregulation in goats. *Am. J. Physiol.* 263 (*Regulatory Integrative Comp. Physiol.* 32): R201–R205, 1992.
23. **Pfaff, D. W.** *Estrogens and Brain Function.* New York: Springer-Verlag, 1980, p. 77–96.
24. **Silva, N. L., and J. A Boulant.** Effects of testosterone, estradiol, and temperature on neurons in preoptic tissue slices. *Am. J. Physiol.* 250 (*Regulatory Integrative Comp. Physiol.* 19): R625–R632, 1986.
25. **Skowsky, W. R., L. Swan, and P. Smith.** Effects of sex steroid hormones on arginine vasopressin in intact and castrated male and female rats. *Endocrinology* 104: 105–108, 1979.
26. **Stricker, E. M., and F. R. Hainsworth.** Evaporative cooling in the rat: effects of dehydration. *Can. J. Physiol. Pharmacol.* 48: 18–27, 1970.
27. **Tankersley, C. G., W. C. Nicholas, D. R. Deaver, D. Mikita, and W. L. Kenney.** Estrogen replacement in middle-aged women: thermoregulatory responses to exercise in the heat. *J. Appl. Physiol.* 73: 1238–1245, 1992.
28. **Turlejska, E., and M. A. Baker.** Aspirin enhances evaporation in hydrated and dehydrated rats. *Can. J. Physiol. Pharmacol.* 66: 72–76, 1988.
29. **Turlejska, E., and M. A. Baker.** Elevated CSF osmolality inhibits thermoregulatory heat loss responses. *Am. J. Physiol.* 251 (*Regulatory Integrative Comp. Physiol.* 20): R749–R754, 1986.
30. **Wilson, N. E., and E. M. Stricker.** Thermal homeostasis in pregnant rats during heat stress. *J. Comp. Physiol. Psychol.* 93: 585–594, 1979.