*Pharmacology Biochemistry & Behavior*, Vol. 18, pp. 563–566, 1983. 

# Ovarian Hormones Influence Brown Adipose Tissue[1]

JOSEPH W. KEMNITZ,[2] ZVI GLICK AND GEORGE A. BRAY[3]

*Division of Endocrinology, Harbor-UCLA Medical Center, Torrance, CA 90509*

Received 21 June 1982

KEMNITZ, J. W., Z. GLICK AND G. A. BRAY. *Ovarian hormones influence brown adipose tissue.* PHARMACOL BIOCHEM BEHAV 18(4) 563–566, 1983.—Adult female rats were ovariectomized (OVX) or sham-operated and 4–5 weeks later OVX groups were treated with estradiol benzoate (EB), progesterone, both hormones, or the oil vehicle. All rats were sacrificed on the 4th day of hormone treatment following an overnight fast and a terminal meal. Interscapular brown adipose tissue (BAT) pads of EB-treated groups were heavier and contained more lipid than those of the other OVX groups. Lipid content of adipose tissue differed according to site (BAT < inguinal < parametrial = retroperitoneal), but only BAT exhibited differential responsiveness to hormonal treatments. There was also a trend for increased oxygen consumption by BAT from EB-treated rats. It is concluded that BAT may be involved in the process of increased energy expenditure by estrogen-treated rats.

Ovariectomy Estradiol Progesterone Energy regulation Brown adipose tissue

ESTROGEN and progesterone have effects on body weight that cannot be entirely accounted for by changes in food intake and locomotion [15]. In general, estrogen decreases body weight and adiposity while progesterone antagonizes these effects of estrogen. However, the weight gain resulting from estrogen withdrawal by ovariectomy is not prevented by restriction of intake to baseline levels [10]. Also, the weight loss of estrogen-treated ovariectomized rats is greater than pair-fed ovariectomized rats, even when the potential contribution of differences in activity is removed [8–10]. Such observations suggest that metabolic processes underlying energy utilization are affected by ovarian hormones.

It has been demonstrated recently that activation of brown adipose tissue (BAT) may provide an additional mechanism for regulating energy balance. For example, the weight and metabolic activity of brown adipose tissue were increased following prolonged overeating [7] or a single meal [1,4]. In these circumstances, substrates may be shunted away from fat stores to brown adipose tissue for oxidation.

The purpose of the present experiment was to explore possible influences of ovariectomy and treatment with estradiol and progesterone on the composition and rate of respiration (*in vitro*) of interscapular BAT following a meal. White adipose tissue from several sites was also assayed for lipid content in order to evaluate differential responsiveness among fat pads [5,14]. Terminal measurements were made four days after beginning hormonal treatments, in time for estrogen- and progesterone-influenced mechanisms to be activated but before adjustments in maintained level of energy store would be expected to be completed [13].

## METHOD

Adult Sprague Dawley rats (Simonsen, Gilroy, CA) were individually caged and given Purina Lab Chow (4.25 Cal/g) and tap water ad lib. The room in which the animals were housed was artificially lighted from 0600 to 1800 hours. Forty-three rats were anesthetized with methohexital (Brevital, Eli Lilly and Co., 32 mg/kg) and either ovariectomized (OVX, n=33) or sham-operated (S, n=10). Beginning 4–5 weeks after surgery the OVX rats were given daily injections of 5 μg estradiol benzoate (EB, Rugby Laboratories, Inwood, NY) or 5 mg progesterone (P; Eli Lilly and Co., Indianapolis, IN) or both hormones (EB + P) or the oil vehicle (V; Mazola Corn Oil). The S rats were given the oil vehicle. Vaginal smears from S rats were microscopically evaluated daily for at least 10 days before sacrifice. On the fourth day of hormone treatment the rats were sacrificed and the completeness of ovariectomy was confirmed visually.

Animals were weighed twice weekly from one week prior to surgery until one week before sacrifice and then daily until sacrifice. Food pellets and tap water were freely available until 18 hours before sacrifice. In addition, a chocolate chip cookie (Chips Ahoy, Nabisco; 4.84 Cal/g) and 16% glucose solution were made available on alternate days for one week before sacrifice. Measurements of body weight and intake of solid food (recorded to the nearest gram), injections of hormones, and evaluations of vaginal smears were accomplished between 0900 and 1100 hours. Beginning 18 hours before sacrifice animals were food deprived for 15 hours. Then three hours before sacrifice they were given a terminal

[1]This research was supported by NIH grants AM27019, AM15156, and RR00167. A preliminary report of this work was presented at the Annual Meeting of the Federation of American Societies for Experimental Biology, New Orleans, LA, 1982.

[2]Requests for reprints should be addressed to Dr. Joseph W. Kemnitz, Wisconsin Regional Primate Research Center, 1223 Capitol Court, Madison, WI 53715-1299.

[3]Present address: Division of Diabetes and Clinical Nutrition, University of Southern California Medical Center, Los Angeles, CA 90033.

TABLE 1
BODY WEIGHT AND FOOD INTAKE

| | Group | | | | |
|---|---|---|---|---|---|
| | S-V | OVX-V | OVX-EB | OVX-EB+P | OVX-P |
| Body Weight (g) | | | | | |
| At surgery | 221 ± 3 | 224 ± 5 | 221 ± 3 | 219 ± 4 | 223 ± 4 |
| At hormone treatment | 248 ± 4 | 286 ± 5 | 295 ± 7 | 284 ± 7 | 283 ± 6 |
| At terminal meal* | 229 ± 3 | 264 ± 4 | 266 ± 6 | 257 ± 7 | 263 ± 6 |
| Food Intake (Cal/4 days) | | | | | |
| Before hormone treatment | 313 ± 10 | 350 ± 8 | 362 ± 13 | 355 ± 13 | 352 ± 11 |
| During hormone treatment | 170 ± 4 | 212 ± 6 | 189 ± 8 | 184 ± 6 | 225 ± 11 |

*After 15-hour deprivation.

TABLE 2
WEIGHT AND COMPOSITION OF FAT PADS

| | Group | | | | |
|---|---|---|---|---|---|
| | S-V | OVX-V | OVX-EB | OVX-EB+P | OVX-P |
| | | | Tissue Weight | | |
| IS-BAT (mg) | 263 ± 10 | 289 ± 11 | 338 ± 21 | 300 ± 12 | 289 ± 23 |
| I-WAT (g) | 1.14 ± 0.08 | 1.50 ± 0.14 | 1.42 ± 0.11 | 1.24 ± 0.09 | 1.46 ± 0.10 |
| PM-WAT (g) | 1.37 ± 0.16 | 1.50 ± 0.21 | 1.34 ± 0.18 | 1.07 ± 0.09 | 1.36 ± 0.14 |
| RP-WAT (g) | 1.09 ± 0.08 | 1.48 ± 0.15 | 1.34 ± 0.12 | 1.17 ± 0.12 | 1.64 ± 0.13 |
| | | | Lipid Content | | |
| IS-BAT (%) | 39 ± 3 | 45 ± 2 | 50 ± 3 | 46 ± 2 | 44 ± 2 |
| I-WAT (%) | 69 ± 2 | 74 ± 2 | 73 ± 2 | 69 ± 3 | 76 ± 1 |
| PM-WAT (%) | 91 ± 1 | 90 ± 1 | 89 ± 1 | 90 ± 1 | 91 ± 1 |
| RP-WAT (%) | 90 ± 1 | 90 ± 1 | 90 ± 1 | 90 ± 2 | 92 ± 1 |
| | | | Nonfat Solids | | |
| IS-BAT (mg) | 48 ± 3 | 50 ± 4 | 45 ± 5 | 44 ± 2 | 45 ± 5 |

meal which consisted of pellets, cookies and 16% glucose. This schedule was followed to ensure that the animals would eat a relatively large meal at a specified time before sacrifice.

Immediately following sacrifice the interscapular brown fat (IS-BAT) and the right inguinal (I), right parametrial (PM) and right retroperitoneal (RP) white adipose tissue (WAT) pads as well as the uteri were carefully dissected out and weighed to the nearest mg. Samples of adipose tissues were frozen for later lipid extraction by a modification of the Folch procedure [3]. The samples of brown fat were also assayed for nonfat solids (NFS) and DNA content [2].

The oxygen consumption of chopped brown fat was measured in a Gilson differential respirometer (Middleton, WI) using a modified Krebs-Ringer bicarbonate buffer containing half of the usual amount of calcium. After a 30-min equilibration period, recordings were made every 10 min for 30 min. Insulin (100 $\mu$U in 0.1 ml) was then added to the medium from the side-arm and, after a 15-min equilibration period, recordings were made every 10 min for 60 min.

Differences among means were evaluated by analysis of variance and subsequent contrasts between pairs of means tested by the Newman-Keuls procedure. When only two groups were being considered, the difference between means was evaluated by Student's $t$-test. Relationships between variables were assessed by the Pearson Product Moment Correlation Coefficient and Student's $t$-test.

## RESULTS

All sham-operated animals exhibited cyclic variations in vaginal cytology during the last two weeks of the experiment. Those animals judged by vaginal cytology to be in proestrous or estrous phases at the time of sacrifice (n=5) had a higher mean uterine wet weight than the remaining rats in the group (481±88 mg vs. 286±52 mg, respectively, $p<0.05$, one-tailed test). Uterine weight also reflected estrogen status among the OVX groups except where attenuated by progesterone: OVX-V, 95±7 mg; OVX-EB, 519±54 mg; OVX-EB+P, 286±19 mg; OVX-P, 126±13 mg.

There were no differences in body weight among groups at the time of surgery, but at the time of hormone treatment and at the time of sacrifice the OVX groups were heavier than the S group ($p<0.01$; Table 1). During the four days of hormone treatment, OVX animals receiving estrogen, either with or without progesterone, exhibited a greater reduction in body weight from pretreatment levels than OVX animals receiving other treatment ($p<0.01$). The two estrogen-treated groups did not differ from each other.

As shown in Table 1, the OVX animals ate more than

TABLE 3
OXYGEN CONSUMPTION OF IS-BAT
(μL/hr/100 mg NONFAT SOLID)

| Condition | Group S-V | OVX-V | OVX-EB | OVX-EB+P | OVX-P |
|---|---|---|---|---|---|
| Basal | 102 ± 16 | 129 ± 21 | 145 ± 23 | 151 ± 20 | 137 ± 23 |
| Insulin Added | 86 ± 10 | 108 ± 18 | 125 ± 17 | 121 ± 12 | 109 ± 12 |

sham-operated animals before and during hormone treatment ($p<0.01$). The OVX-EB and OVX-EB+P groups ate less than the OVX-V and OVX-P groups during the four days before sacrifice ($p<0.01$). Intake of solid food during the terminal meal averaged 33±2 Cal overall, while intake of glucose solution contributed an additional 6±1 Cal overall.

The mean weight of brown fat pads from the S group was less than that of each of the other groups ($p<0.05$), while the mean weight of brown fat from the OVX-EB group was greater than that of the others ($p<0.01$; Table 2). Variance in lipid content of BAT accounted for 68% of the variance in BAT weight ($r=+0.83$, $p<0.01$) across groups. The OVX-EB group had the greatest lipid content of BAT ($p<0.01$) and the S group had less lipid in BAT than any other group ($p<0.01$). The OVX-EB+P group had marginally greater lipid content in BAT than the OVX-V and OVX-P groups ($p<0.05$). Within the sham-operated group those rats judged to be in proestrus or estrus had 22% more lipid in their brown fat than the remaining animals in that group, but this trend did not achieve statistical significance. The brown fat did not differ among groups in the quantity of nonfat solids or DNA.

Lipid content of adipose tissue was highly correlated with pad weight for all groups and for all sites ($r>+0.83$; Table 2). The brown fat contained the least lipid (44±1% overall) and the parametrial and retroperitoneal white fat the most lipid (90±1% overall), while the inguinal fat pad was intermediate (72±1% overall).

Oxygen consumption of BAT in the basal and insulin-stimulated conditions was highly correlated ($r=+0.96$), and the respiration rate after insulin was added was approximately 20% lower than in the basal condition ($p<0.01$; Table 3). There was a trend for the EB-treated groups to have greater oxygen consumption, but these differences were not statistically significant. Oxygen consumption was not detectably related to lipid content of IS-BAT or to the size of the terminal meal.

## DISCUSSION

Short-term treatment of ovariectomized rats with estrogen led to an increase in brown fat weight. This increase in weight was attributable to an increase in the lipid content of the tissue. Rats with intact gonads studied during the time of the estrous cycle when estrogen is elevated [11] also exhibited a trend towards increased weight and lipid content of brown fat. This increased rate of lipid accumulation in BAT occurred in the face of EB-induced suppression of food intake and reflected a net increase in the rate of lipid synthesis in this tissue. An increased rate of lipogenesis in BAT has been shown to be closely associated with increased BAT thermogenesis [12]. The present data therefore suggest that the increased energy expenditure of estrogen-treated rats may be mediated by BAT.

Ovariectomized rats generally had more fat than intact rats in all sites sampled. Also, across all groups, the intra-abdominal sites (parametrial and retroperitoneal) contained more lipid per gram than the subcutaneous sites (inguinal and interscapular). The BAT had the lowest lipid content of the four sites studied.

Receptors for estradiol and progestins have been identified in cytoplasm of brown fat as well as white fat and the binding capacity of these receptors differs according to tissue site [5,14]. This suggests that ovarian hormones may directly influence metabolic processes in adipocytes and that various sites may be differentially sensitive to the effects of ovarian hormones. The present observation that changes in lipid content of BAT but not WAT occurred after estrogen treatment suggests that BAT may be particularly responsive to direct estrogenic influences on metabolism. It is also possible, however, that the effect of estrogen on brown fat may be neurally mediated.

Although estrogen treatment tended to increase meal-induced thermogenesis in BAT, this trend was not statistically significant. Since BAT thermogenesis is increased 2–3 fold after a meal [4], it is possible that an estrogen-induced increase in thermogenesis may have been masked by the large, meal-induced changes. Additional studies of the regulation of BAT metabolism would be useful for achieving better understanding of mechanisms for the regulation of energy balance and their modulation by ovarian hormones.

## ACKNOWLEDGEMENTS

We are grateful to Dr. J. Fisler and Mrs. M. Lee for advice and technical assistance and to Mrs. M. Schatz and Mrs. J. Kinney for secretarial assistance. Dr. S. Pomerantz provided helpful comments regarding the preparation of the manuscript. This is publication number 22-019 of the Wisconsin Regional Primate Research Center.

## REFERENCES

1. Agius, L. and D. H. Williamson. Lipogenesis in interscapular brown adipose of virgin, pregnant and lactating rats. *Biochem J* **190:** 477–480, 1980.
2. Burton, K. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonuleic acid. *Biochemistry* **62:** 315–323, 1956.
3. Folch, J., M. Lees and G. H. Sloane-Stanley. A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem* **226:** 497–509, 1957.
4. Glick, Z., R. J. Teague and G. A. Bray. Brown adipose tissue: Thermic response increased by a single low protein, high carbohydrate meal. *Science* **213:** 1125–1127, 1981.

5. Gray, J. M. and G. N. Wade. Cytoplasmic progestin binding in rat adipose tissues. *Endocrinology* **104:** 1377–1382, 1979.
6. Hervey, E. and G. R. Hervey. Energy storage in female rats treated with progesterone in the absence of increased food intake. *J Physiol* **200:** 118P–119P, 1968.
7. Rothwell, N. J. and M. J. Stock. A role for brown adipose tissue in diet-induced thermogenesis. *Nature* **281:** 31–35, 1979.
8. Roy, E. J. and G. N. Wade. Role of estrogens in androgen-induced spontaneous activity in male rats. *J Comp Physiol Psychol* **89:** 573–579, 1975.
9. Roy, E. J. and G. N. Wade. Estrogenic effects of an antiestrogen, MER-25, on eating and body weight in rats. *J Comp Physiol Psychol* **90:** 156–166, 1975.
10. Roy, E. J. and G. N. Wade. Role of food intake in estradiol-induced body weight changes in female rats. *Horm Behav* **8:** 255–274, 1977.
11. Schwartz, N. B. Acute effects of ovariectomy on pituitary LH, uterine weight, and vaginal cornification. *Am J Physiol* **207:** 1251–1259, 1964.
12. Trayhurn, P. Fatty acid synthesis *in vivo* in brown adipose tissue, liver, and white adipose tissue on the cold-acclimated rat. *FEBS Lett* **104:** 13–16, 1979.
13. Wade, G. N. Some effects of ovarian hormones on food intake and body weight in female rats. *J Comp Physiol Psychol* **88:** 183–193, 1975.
14. Wade, G. N. and J. M. Gray. Cytoplasmic 17 $\beta$-[$^3$H] estradiol binding in rat adipose tissues. *Endocrinology* **103:** 1695–1701, 1978.
15. Wade, G. N. and J. M. Gray. Gonadal effects on food intake and adiposity: A metabolic hypothesis. *Physiol Behav* **22:** 583–593, 1979.