Biochemical and Biophysical Research Communications **288,** 191–197 (2001)
doi:10.1006/bbrc.2001.5763, available online at http://www.idealibrary.com on IDEAL®

Regulation of UCP1, UCP2, and UCP3 mRNA Expression in Brown Adipose Tissue, White Adipose Tissue, and Skeletal Muscle in Rats by Estrogen

Steen B. Pedersen,*,†,[1] Jens M. Bruun,* Kurt Kristensen,* and Bjørn Richelsen*,†
**Department of Endocrinology and Metabolism C, Aarhus Amtssygehus, Aarhus University Hospital, Aarhus, Denmark; and †Faculty of Health Sciences, Aarhus University, Aarhus, Denmark*

Received September 5, 2001

The effects of ovariectomy (OVX) and estrogen substitution on body weight, body composition, food intake, weight gain, and expression of uncoupling proteins (UCPs) in brown adipose tissue (BAT), white adipose tissue (WAT), and skeletal muscle were studied in four groups of rats: (1) Sham-operated rats ($N = 8$), (2) ovariectomized rats (OVX − E) ($N = 8$), (3) estrogen-treated OVX rats (OVX + E) ($N = 8$), and (4) OVX rats on energy restriction (OVX − E + D) ($N = 8$). OVX was associated with an increase in food intake and body weight gain during a 5-week study period compared to sham-operated rats. The estrogen-substituted rats had a significantly lower food intake and weight gain during the 5 weeks compared to the sham-operated group. However, we also included a nontreated OVX group that was allowed to eat only enough chow to match the weight gain of the sham-operated group. To match the weight gain in the two groups, the OVX group had to consume 16% less chow than the sham-operated group. In BAT, the UCP1 expression was significantly lower in estrogen-deficient rats compared to either intact rats or estrogen-substituted rats, whereas UCP2 and UCP3 mRNA expression was similar in BAT from all four groups. In WAT, both estrogen-deficient groups had significantly lower UCP2 mRNA expression compared to the control rats and estrogen-treated rats; In contrast, the UCP3 mRNA expression in WAT was similar in all four groups. Finally, in skeletal muscle the OVX group on mild energy restriction had reduced UCP3 mRNA expression compared to control, OVX, and estrogen-treated rats. In contrast, the UCP2 mRNA expression in skeletal muscle was similar in all four groups. Thus, the findings that estrogen deficiency is followed by reduced UCP1 expression in BAT and reduced UCP2 expression in WAT in association with weight gain probably caused by a decrease in energy expenditure might indicate that UCPs play a role for the estrogen-mediated changes in body weight and energy expenditure. © 2001 Academic Press

Key Words: **estrogen; adipose tissue; skeletal muscle; brown adipose tissue; UCP; food intake; body weight.**

Ovarian hormones are among the many factors influencing body weight in mammals. Estradiol given to ovariectomized animals depress eating (1, 2) and in addition the animals tend to be more physically active compared to estradiol-deprived animals (1, 3). Thus, both these effects could explain the retarded weight gain observed in estradiol-treated animals compared with ovariectomized animals. However, other studies have shown that estrogen also seems to affect body weight regulation/energy expenditure through other pathways than food intake and physical activity (4, 5). Supporting this notion are the studies demonstrating that estradiol treatment is associated with an increase in oxygen consumption, indicating an elevated energy expenditure (6). Recently, the importance of estrogen for the body composition/energy expenditure was strengthened by demonstrating that estrogen receptor knockout mice had much more white adipose tissue than wild-type mice; in addition, it was shown that the knockout mice had an 11% decrease in energy expenditure (7). The precise nature of this increase in energy expenditure is, however, unknown. Bartness and Wade demonstrated that the brown adipose tissue (BAT) and the innervation to this tissue was very important for the estrogen mediated changes in energy expenditure, as denervation of BAT greatly markedly impaired the estrogen-induced increase in oxygen consumption (8). In rodents BAT is probably the most important tissue in regulation of energy expenditure, and BAT is able to increase the heat production several fold, by uncoupling the electron transport chain from ATP formation

[1] To whom correspondence should be addressed at Department of Endocrinology and Metabolism C, Aarhus Amtssygehus, Aarhus University Hospital, DK-8000 Aarhus C, Denmark. Fax: 89497649. E-mail: amtssp@aau.dk.

0006-291X/01 $35.00

in the mitochondria, and instead energy is dissipated as heat. The molecular mechanism for this uncoupling in BAT is predominantly caused by the uncoupling protein 1 (UCP1) which is located in the inner mitochondrial membrane (9). UCP1 shortcuts the proton gradient, whereby the proton transport is uncoupled from the ATP production. Recently two new members of the UCP family have been discovered, the UCP2 which is present in most tissues and the UCP3 which is particularly expressed in skeletal muscle (10, 11). These new UCPs are thought to function as uncouplers in different tissues in the same manner as UCP1 in BAT.

Because the UCPs might influence energy expenditure, and because the precise molecular basis for the estrogen-mediated effect on energy homeostasis is largely unknown, we decided to study the regulation of UCPs in BAT as well as in adipose tissue and skeletal muscle in ovariectomized rats, treated and untreated with estrogen.

MATERIALS AND METHODS

Female Wistar rats were ovariectomized under pentobarbital anaesthesia using a midline incision another eight rats were not ovariectomized but sham operated (control rats). The ovariectomized rats were divided into three groups, one group (OVX + E) ($N = 8$) received a subcutaneous pellet implantation containing a slow release 17-β-estradiol pellet (1.5 mg/pellet, 60-day release) (Innovative Research of America ,FL), another OVX group was left untreated (OVX − E) ($N = 8$). In the last group the OVX rats did not receive estrogen but were on a hypocaloric diet (OVX − E + D) ($N = 8$), where the food intake was adjusted so the rats in this group had the same weight gain as the control group. This group was included in the study in order to discriminate between effects induced by estrogen compared to effects associated with body weight changes per se.

The rats were housed in temperature (20–22°C) and light controlled (12/12 h light–dark cycle) environment. They had access to a standard chow and water. To properly match the weight gain in the OVX − E + D group with that of the control group, the food consumption and body weight were measured daily in the control group and in the OVX − E + D group. After a few days it turned out that a ~15% daily reduction in the offered food resulted in a weight gain in the OVX − E + D group similar to that of the control rats.

Body composition: Bioelectrical impedance measurements in rats. At the end of the study the rats were anaesthetized using pentobarbital, and bioelectrical impedance was measured using a multifrequency bioimpedance spectroscopy analyzer (SFB3, UniQuest Limited, Brisbane, Australia) as previously described by Cornish *et al.* (12). The following equation was used for calculation of the fat-free mass (FFM) = $(131.2981 \cdot L^2/Zc + 37.3184)/0.732$, assuming a hydration of 0.732 (Ward *et al.*, personal communication). Fat mass (FM) = body weight − FFM.

Removal of BAT, white adipose tissue, and skeletal muscle. Immediately after sacrifice the abdomen was opened and the parametrial and perirenal adipose tissue was carefully dissected and weighted separately, Then the adipose tissue was snap-frozen in liquid nitrogen, and stored at −80°C for later analysis.

The soleus muscle and the brown adipose tissue were carefully removed, snap-frozen in liquid nitrogen, and stored at −80°C for later analysis.

RNA isolation. Total RNA was isolated from the biopsies using the TriZol reagent (Gibco BRL, Life Technologies, Roskilde, Denmark), RNA was quantitated by measuring absorbency at 260 and 280 nm. Finally, the integrity of the RNA was checked by visual inspection of the two ribosomal RNA's 18S and 28S on an agarose gel.

Real time RT-PCR detection of UCP mRNA expression. cDNA was made using random hexamer primers as described by the manufacturer (GeneAmp RNA PCR Kit from Perkin–Elmer Cetus, Norwalk, CT). Then, PCR mastermix containing the specific primers and AmpliTaq Gold DNA polymerase were added.

UCP1-primers: 5′-GTG AGT TCG ACA ACT TCC GAA GTG and 5′-CAT CAG GTC ATA TGT CAC CAG CTC. UCP2-primers: 5′-TTCAAGGCCACAGATGTGCC and 5′-TCGGGCAATGGTCTTGTAGGC. UCP3 primers: 5′-CCCTGACTCCTTCCTCCCTG and 5′-GCACTGCAGCCTGTTTTGCTGA, β-actin primers: 5′-TGTGCCCATCTACGAGGGGTATGC and 5′-GGTACATGGTGGTGCCGCCAGACA.

Real time quantitation of UCP1, UCP2, and UCP3 to β-actin mRNA was performed with a SYBR-Green real-time PCR assay using an ICycler PCR machine from Bio-Rad. Briefly, UCP1, UCP2, UCP3, and β-actin mRNA were amplified in separate tubes using the following protocol 95°C in 10 min, then each cycle comprising 95°C for 30 s, 57°C for 30 s and extension at 72°C for 60 s, the increase in fluorescence was measured in real-time during the extension step. The threshold cycle (C_t) which is defined as the fractional cycle number at which the fluorescence reaches 10× the standard deviation of the baseline was calculated, and the relative gene expression was calculated essentially as described in the User Bulletin 2, 1997 from Perkin–Elmer (Perkin–Elmer Cetus, Norwalk, CT) covering the aspect of relative quantitation of gene expression. Briefly the target gene (X_0) to β-actin (R_0) ratio in each sample before amplification was calculated as $X_0/R_0 = k \times 1/((2)^{**} \delta C_t)$, δC_t is the difference between C_t target and C_t reference, and k is a constant, set to 1.

All samples were amplified in duplicate. A similar setup was used for negative controls except that the reverse transcriptase was omitted and no PCR products were detected under these conditions

Statistically analysis. Differences between group means were determined using ANOVA with Duncan's post hoc analysis. Data are presented as means ± SEM, and a significance level below 0.05 was chosen.

RESULTS

Effects of OVX and Estrogen Substitution on Body Weight (Fig. 1)

The mean body weights of the rats in the four groups are outlined in Fig. 1. At the time of operation all rats had similar body weight (164.6 ± 5.0 g) and the control group gained around 50 g during the 5-week study period reaching a mean weight of 220.0 ± 10.2 g after the 5 weeks. The OVX − E rats gained significantly more and after five weeks the mean weight in this group was 260.3 ± 11.4 g, whereas, the OVX + E rats gained much less (final weight 196.0 ± 15.3 g) compared both to the control group and the OVX − E group. Finally, the group of nonsubstituted OVX rats, that were on energy restriction (OVX − E + D) obtained a weight (220.1 ± 6.8 g) similar to that of the control rats (Fig. 1).

Effects of OVX and Estrogen Substitution on Food Intake (Fig. 2)

The food intake in all rats was measured two times during the study, after 3 weeks and after 5 weeks. As

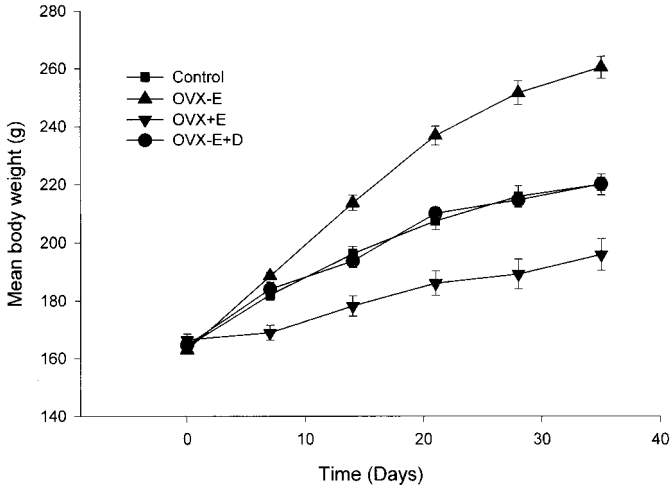

FIG. 1. The body weight of the rats in the four different groups were followed weekly. Control, sham operated; OVX + E, ovariectomized and estrogen substituted; OVX − E, ovariectomized no estrogen treatment; and OVX − E + D, ovariectomized no estrogen treatment but on diet restriction.

shown in Fig. 2, the control rats ate 19.98 ± 0.31 g/rat/24 h 3 weeks after surgery, whereas OVX − E rats consumed significantly more (23.38 ± 0.33 g/rat/24 h) and estrogen treated (OVX + E) rats consumed significantly less than the control rats (17.68 ± 0.39 g/rat/24 h). The OVX − E + D rats on the restricted diet which had a weight gain similar to the control rats was given significantly less food (18.50 ± 0.29 g/rat/24 h) ($P < 0.05$) than the control rats.

Almost identical findings were obtained after 5 weeks; however, the OVX − E rats now consumed

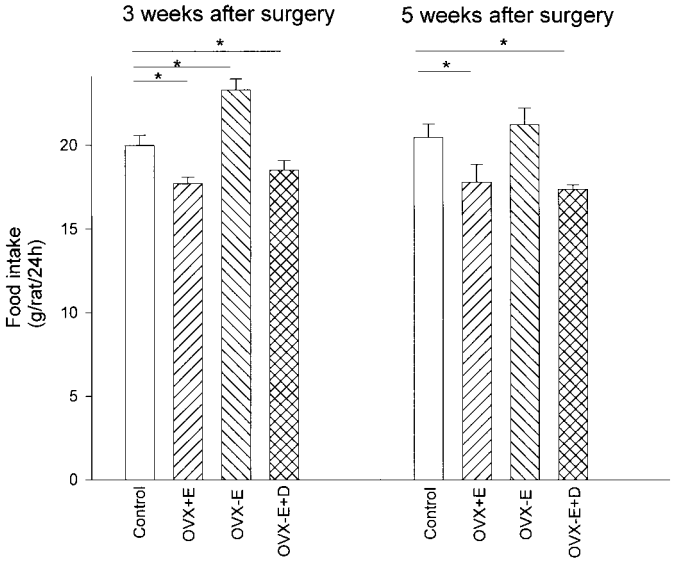

FIG. 2. The food intake was measured twice during the experiment. 3 weeks after the start, and 5 weeks after the start of the experiment. (*$P < 0.05$, ANOVA followed by Duncan's post hoc analysis).

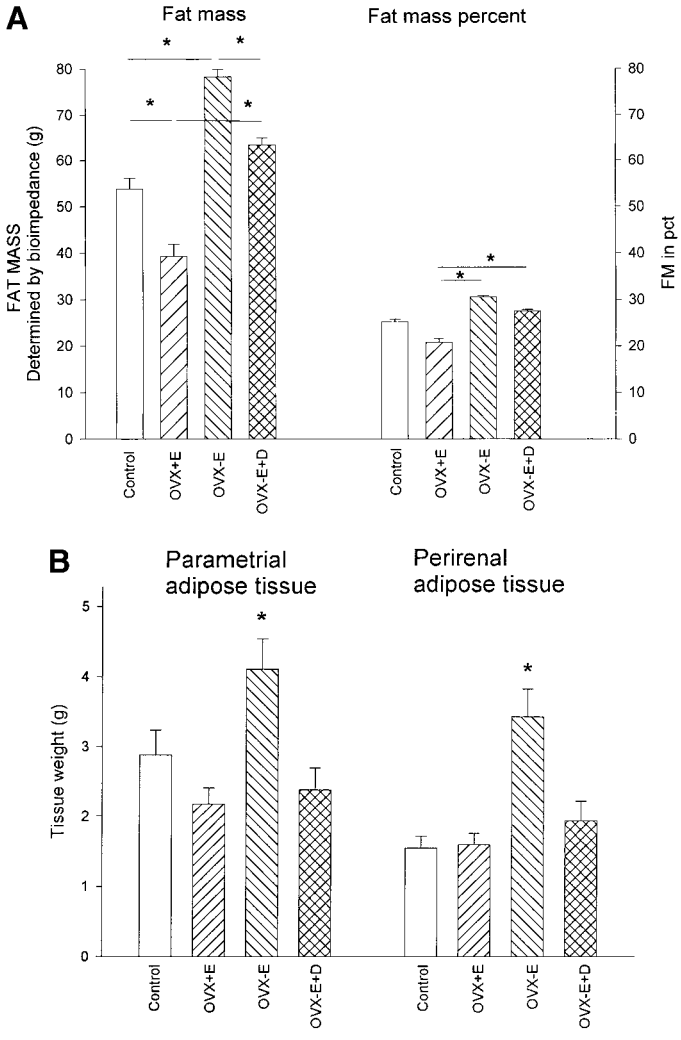

FIG. 3. (A) Bioimpedance determined body composition: Left-hand panel shows the amount of adipose tissue in each of the four groups of rats. Right-hand panel: The amount of adipose tissue as percent of body weight. (B) The weight of the adipose tissue pads (*$P < 0.05$, ANOVA followed by Duncan's post hoc analysis).

significantly less than they did earlier in the study. In contrast, the food intake of the estrogen-substituted (OVX + E) rats and the control rats did not change during the study period.

Effects of OVX and Estrogen Substitution on Body Composition (Fig. 3)

The body composition was determined at the end of the study by impedance measurements and dissection of the adipose tissue.

Impedance measurements showed that the absolute amount of adipose tissue was significantly increased in the OVX − E rats compared to both the control rats and the OVX − E + D rats. The estrogen substituted OVX + E rats had significantly less adipose tissue than the control rats (Fig. 3A). The OVX − E + D rats (the

rats on energy restriction) had an insignificant increase in amount of adipose tissue compared to sham-operated rats and were significantly heavier than the estrogen-treated rats (OVX + E) (Fig. 3A).

If the FM% was calculated (100 × fat mass (g)/body weight (g)), the estrogen-treated group (OVX + E) and the control group had similar FM%. In contrast the FM% in the estrogen treated rats were significantly lower than in the two estrogen deficient groups (OVX − E and OVX − E + D) (Fig. 3A).

The weight of the dissected parametrial adipose tissue and the perirenal adipose tissue are shown in Fig. 3B. The OVX − E rats had significantly increased amount of adipose tissue in both regions ($P < 0.001$), whereas, the three other groups had a comparable amount of adipose tissue (Fig. 3B).

UCP mRNA Expression in Brown Adipose Tissue

As shown in Fig. 4A, the expression of UCP1 in BAT compared with control rats was significantly lower in rats devoid of estrogen (OVX − E rats and OVX − E + D), whereas OVX + E rats had an UCP1 expression comparable to that of control rats.

Moreover, the UCP2 expression in BAT changed in a similar manner as UCP1, with a reduction in estrogen-deficient rats (however, this did not reach statistical significance). The UCP3 expression in BAT did not differ among the four groups.

White Adipose Tissue

The UCP2 mRNA expression was significantly reduced in the two estrogen-deficient groups (OVX − E and OVX − E + D), whereas the OVX + E group had an UCP2 expression in adipose tissue comparable to that of the control group (Fig. 4B). The UCP3 mRNA expression in adipose tissue was similar in all four groups (Fig. 4B). Finally, the UCP1 expression in WAT was also investigated; however, no reproducible amplification was obtained even after 50 PCR cycles.

Skeletal Muscle

The UCP2 mRNA expression was similar in all four groups (Fig. 4C), whereas, the UCP3 mRNA expression was significantly reduced in the energy-restricted group (OVX − E + D) (Fig. 4C) compared with the other groups ($P < 0.05$). The UCP1 expression was also evaluated in skeletal muscle, however, no UCP1 mRNA was detected.

DISCUSSION

Shortly after ovariectomy the rats begin to overeat which is followed by a concomitant increase in body weight and adiposity, and all these changes can be reverted by estrogen treatment as also previously described (1, 2). Estrogens have previously been shown to affect food intake, as also demonstrated in the present study, therefore the changes in body weight might just be secondarily to changes in food intake. However, by the inclusion of an energy-restricted OVX group, we are able to distinguish between those effects on body weight that are mediated through the effects of estrogens on food intake, and those effects of estrogen that are separate from food intake. When analyzing the rats on energy restricted diet we found that in order to match the weight gain in sham-operated rats with free access to chow, we had to reduce the food intake in OVX rats to 84% of the sham-operated rats, indicating that the caloric efficiency was higher in estrogen deficient rats, these findings are well in line with the findings of Roy and Wade (5) showing that OVX rats given a mean food ration of 80% of the *ad libitum* intake in sham operated rats prevented the post-OVX obesity to develop.

Many studies support the notion, that estrogen treatment is associated with increased oxygen consumption (energy expenditure) (6, 8, 13, 14). The reason for the increase in energy production is not settled, however, some studies suggest that estrogen treatment is associated with an increase in heat loss (6, 15, 16). In addition estrogen treated rats have increased behavioral activity (17)) which also tend to increase the energy expenditure. However, Laudenslager *et al.* (6) elegantly demonstrated that estrogen treatment of ovariectomized rats was associated with increased heat production even in restrained rats; furthermore, the rats were shaved in order to equalize for any possible differences in insulation (fur thickness) (6). In addition in the study by Roy and Wade (5) a nonsteroidal anti-estrogen (MER-25) was used, which is fully estrogenic for food intake. Thus, it depresses food intake and body weight gain similar to that of estrogen; however, this compound does not stimulate voluntary exercise (3), demonstrating that estrogens can cause weight loss independent of an increase in exercise. Thus, it seems that other mechanisms than decreased food intake and increased voluntary exercise affect body-weight change in estrogen-treated OVX rats.

The present study demonstrates that estrogen deficient rats (OVX − E + D) were more food-efficient because they had to consume 16% less chow than sham-operated control rats in order for the two groups to obtain similar weight gain. In addition, the estrogen-deficient rats on energy restriction (OVX − E + D) which actually consumed the same amount of food as the estrogen treated rats (OVX + E) had a higher weight gain and contained more adipose tissue than the OVX + E rats. These findings are very suggestive of a lower energy expenditure in the estrogen deficient rats, which is also suggested in a recent paper studying estrogen receptor α knockout mice (7). The knockout mice demonstrated an 11% reduction in energy expen-

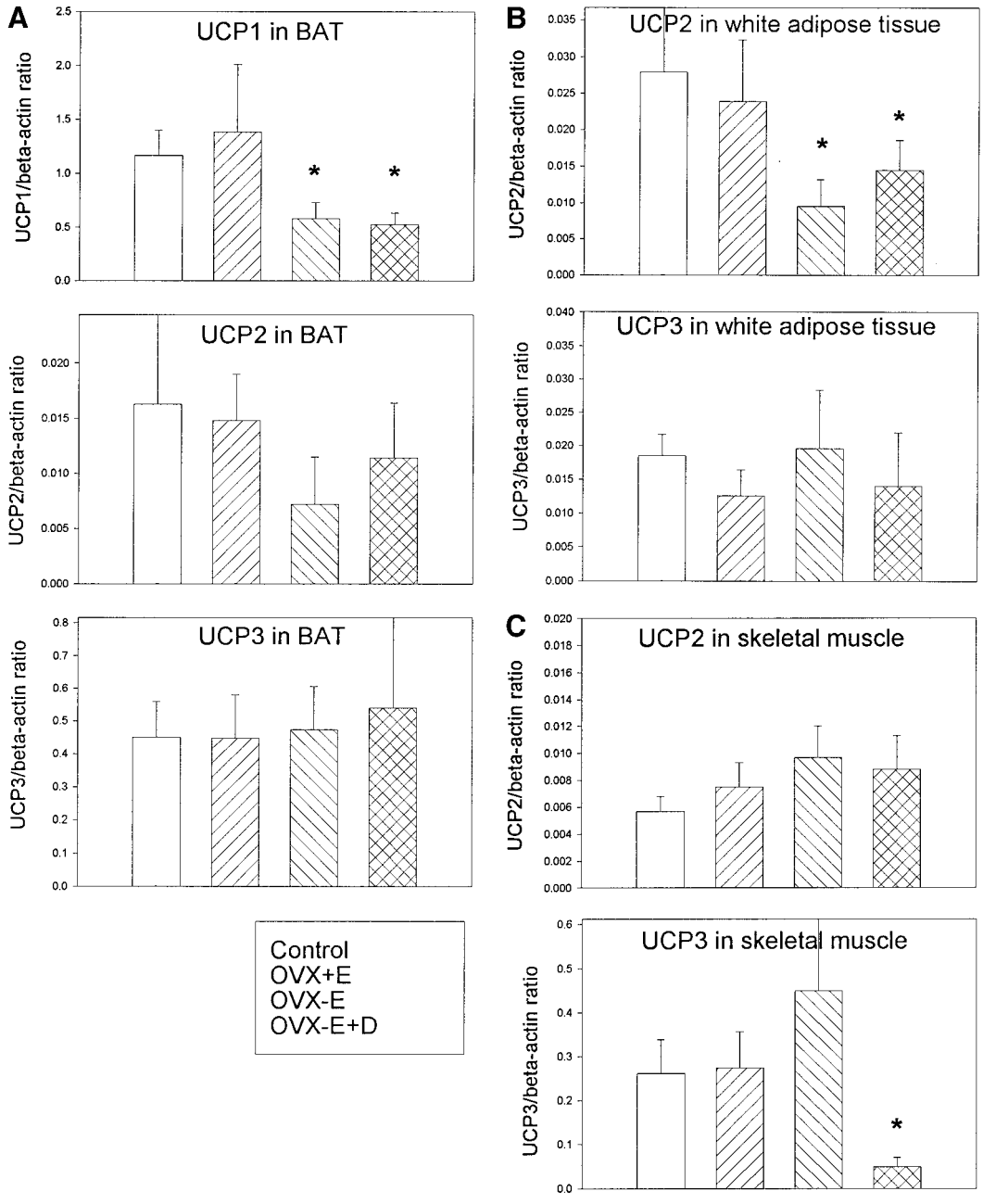

FIG. 4. (A) Expression of UCPs in BAT. The UCP1, UCP2 and UCP3 mRNA to β-actin mRNA expression were calculated as stated in materials and methods section. (B) Expression of UCP2 and UCP3 mRNA to β-actin mRNA in white adipose tissue. (C) Expression of UCP2 and UCP3 mRNA to β-actin mRNA in skeletal muscle (*, $P < 0.05$, ANOVA followed by Duncan's post hoc analysis).

diture compared to wild-type mice; accordingly, they became more obese than their wild-type littermate even though the food intake was similar in the two groups (7). This notion is substantiated in our present study by the demonstration that the expression of the uncoupling proteins in different tissues are regulated by estrogens. In BAT, which probably is the most important tissue for regulation of energy expenditure in rodents, the UCP1 expression in estrogen deficient rats was significantly lower than that of the estrogen-treated OVX rats (OVX + E) or control rats. In 1984 Bartness *et al.* (8) described that estrogen treatment of OVX rats was associated with increased oxygen consumption, but if BAT was denervated the increase in oxygen consumption after estrogen treatment could be blocked, indicating that the BAT is an important tissue for the estrogen effects on energy expenditure and that an intact innervation is important for the estrogen effects on energy expenditure. In a recent publication the regulation of UCP1 in BAT was investigated dem-

onstrating that female rats had significantly higher UCP1 expression in BAT (18), in contrast they reported that the incubation of cultured BAT-precursor cells with estradiol actually decreased the norepinephrine stimulated UCP1 expression. Thus there seems to be conflicting results on the effects of estrogen on BAT *in vivo* compared to *in vitro*, which might indicate that the effects on BAT are indirect and might involve activation of the sympathetic nervous system.

Furthermore, we found that the UCP2 expression in adipose tissue was significantly lower in estrogen deficient rats and the reduction in UCP2 mRNA expression could be normalized by estrogen treatment.

Finally, the OVX group that was on food-restriction (OVX − E + D) had a significant lower UCP3 mRNA expression than the OVX − E group, and also lower than the two other groups (control and OVX + E). These findings indicate that skeletal muscle UCP expression is not regulated by estrogens, whereas, mild food restriction seems to down regulate UCP3 mRNA expression, a finding which is well in line with the findings of Boss *et al.* (19) showing that moderate food-restriction is associated with a decrease in skeletal muscle UCP3 expression.

From the present study it seems clear that estrogens can modulate the expression of UCP2 in white adipose tissue; however, the exact nature of this regulation is still not settled. Adipose tissue contains both the classical estrogen receptor α (20–22) but also the newly described estrogen receptor β (23, 24), thus, it is possible that estrogen can influence the UCP2 expression directly through binding to one of these estrogen receptors. However, it is also possible that the observed effects on UCP expression are caused indirectly by estrogen, possibly by estrogen affecting the sympathetic nervous system (25) or through changes in other hormones/metabolites.

In conclusion the uncoupling proteins in adipose tissue (UCP1 in BAT and UCP2 in WAT) seem to be downregulated in estrogen-deficient rats, and normalize after estrogen substitution. The pattern of regulation of the UCPs is in accordance with the well-known pattern of energy expenditure in OVX rats. Thus, our findings might indicate of a role for these proteins in the regulation of energy expenditure by estrogen in rats.

REFERENCES

1. Wade, G. N. (1972) Gonadal hormones and behavioral regulation of body weight. *Physiol. Behav.* **8,** 523–534.
2. Wade, G. N. (1975) Some effects of ovarian hormones on food intake and body weight in female rats. *J. Comp. Physiol. Psychol.* **88,** 183–193.
3. Roy, E. J., and Wade, G. N. (1975) Role of estrogens in androgen-induced spontaneous activity in male rats. *J. Comp. Physiol. Psychol.* **89,** 573–579.
4. Landau, I. T., and Zucker, I. (1976) Estrogenic regulation of body weight in the female rat. *Horm. Behav.* **7,** 29–39.
5. Roy, E. J., and Wade, G. N. (1977) Role of food intake in estradiol-induced body weight changes in female rats. *Horm. Behav.* **8,** 265–274.
6. Laudenslager, M. L., Wilkinson, C. W., Carlisle, H. J., and Hammel, H. T. (1980) Energy balance in ovariectomized rats with and without estrogen replacement. *Am. J. Physiol.* **238,** R400–R405.
7. Heine, P. A., Taylor, J. A., Iwamoto, G. A., Lubahn, D. B., and Cooke, P. S. (2000) Increased adipose tissue in male and female estrogen receptor-alpha knockout mice [in process citation]. *Proc. Natl. Acad. Sci. USA* **97,** 12729–12734.
8. Bartness, T. J., and Wade, G. N. (1984) Effects of interscapular brown adipose tissue denervation on body weight and energy metabolism in ovariectomized and estradiol-treated rats. *Behav. Neurosci.* **98,** 674–685.
9. Bouillaud, F., Ricquier, D., Mory, G., and Thibault, J. (1984) Increased level of mRNA for the uncoupling protein in brown adipose tissue of rats during thermogenesis induced by cold exposure or norepinephrine infusion. *J. Biol. Chem.* **259,** 11583–11586.
10. Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M. F., Surwit, R. S., Ricquier, D., and Warden, C. H. (1997) Uncoupling protein-2: A novel gene linked to obesity and hyperinsulinemia. *Nat. Genet.* **15,** 269–272.
11. Boss, O., Samec, S., Paoloni-Giacobino, A., Rossier, C., Dulloo, A., Seydoux, J., Muzzin, P., and Giacobino, J. P. (1997) Uncoupling protein-3: A new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett.* **408,** 39–42.
12. Cornish, B. H., Thomas, B. J., and Ward, L. C. (1993) Improved prediction of extracellular and total body water using impedance loci generated by multiple frequency bioelectrical impedance analysis. *Phys. Med. Biol.* **38,** 337–346.
13. Carlisle, H. J., Wilkinson, C. W., Laudenslager, M. L., and Keith, L. D. (1979) Diurnal variation of heat intake in ovariectomized, steroid-treated rats. *Horm. Behav.* **12,** 232–242.
14. Marrone, B. L., Gentry, R. T., and Wade, G. N. (1976) Gonadal hormones and body temperature in rats: Effects of estrous cycles, castration and steroid replacement. *Physiol. Behav.* **17,** 419–425.
15. Laudenslager, M. L., Carlisle, H. J., and Calvano, S. E. (1982) Increased heat loss in ovariectomized hypothyroid rats treated with estradiol. *Am. J. Physiol.* **243,** R70–R76.
16. Fregly, M. J., Kelleher, D. L., and Black, D. J. (1979) Tolerance of estrogen-treated rats to acute cold exposure. *J. Appl. Physiol.* **47,** 59–66.
17. Stern, J. J., and Murphy, M. (1972) The effects of thyroxine and estradiol benzoate on wheel running activity in female rats *Physiol. Behav.* **9,** 79–82.
18. Quevedo, S., Roca, P., Pico, C., and Palou, A. (1998) Sex-associated differences in cold-induced UCP1 synthesis in rodent brown adipose tissue. *Pflugers Arch.* **436,** 689–695.
19. Boss, O., Samec, S., Kuhne, F., Bijlenga, P., Assimacopoulos-Jeannet, F., Seydoux, J., Giacobino, J. P., and Muzzin, P. (1998) Uncoupling protein-3 expression in rodent skeletal muscle is modulated by food intake but not by changes in environmental temperature. *J. Biol. Chem.* **273,** 5–8.
20. Wade, G. N., and Gray, J. M. (1978) Cytoplasmic 17 β-[^{3}H]estradiol binding in rat adipose tissues. *Endocrinology* **103,** 1695–1701.
21. Gray, J. M., Dudley, S. D., and Wade, G. N. (1981) *In vivo* cell nuclear binding of 17β-[^{3}H]estradiol in rat adipose tissues. *Am. J. Physiol.* **240,** E43–E46.
22. Pedersen, S. B., Borglum, J. D., Eriksen, E. F., and Richelsen, B. (1991) Nuclear estradiol binding in rat adipocytes. Regional vari-

ations and regulatory influences of hormones. *Biochim. Biophys. Acta* **1093,** 80–86.

23. Crandall, D. L., Busler, D. E., Novak, T. J., Weber, R. V., and Kral, J. G. (1998) Identification of estrogen receptor beta RNA in human breast and abdominal subcutaneous adipose tissue. *Biochem. Biophys. Res. Commun.* **248,** 523–526.
24. Pedersen, S. B., Bruun, J. M., Hube, F., Kristensen, K., Hauner, H., and Richelsen, B. (2001) Demonstration of estrogen receptor subtypes alpha and beta in human adipose tissue: Influences of adipose cell differentiation and fat depot localization. *Mol. Cell Endocrinol.* **182,** 27–37.
25. Lazzarini, S. J., and Wade, G. N. (1991) Role of sympathetic nerves in effects of estradiol on rat white adipose tissue. *Am. J. Physiol.* **260,** R47–R51.