Metabolic effects of physical training in ovariectomized and hyperestrogenic rats

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It is now well established that ovariectomy (OVX) in rats leads to increases in food intake and body weight, whereas estradiol treatment abolishes these effects (14, 15, 20). Although the results have been somewhat conflicting, several studies have also reported overall insulin resistance in experimental animals after OVX, and these effects can be partially reversed by hormone replacement (3, 13, 19). On the other hand, exercise training in rats has repeatedly been reported to be associated with an enhanced insulin sensitivity (4, 5, 18). In this context, it is of interest to determine how OVX animals adapt to endurance exercise training and if OVX animals can benefit from an exercise training program. The only existing study on this matter was specific to skeletal muscle, showing that OVX had no effect on the increases in skeletal muscle oxidative enzyme activities and glucose transporter concentration elicited by a swim-training protocol (11). One of the purposes of the present experiment was to investigate the effects of ovarian hormone deficiency on the effects of regular endurance-type exercise regarding changes in body weight and whole body glucose tolerance and glucose-stimulated insulin response (GSIR).

In addition to being associated with termination of reproductive life in women, menopause coincides with an increase in several comorbidities, including cardiovascular disease and its associated components, such as body fatness and insulin resistance (for a review, see Ref. 24). Therapies aimed at preventing these changes in women include hormone-replacement therapy, diet, and physical exercise (24). It is, therefore, of interest to determine how estrogen replacement interacts with physical training. The sparse existing studies on this matter indicate that exercise training and estrogen replacement interact to prevent tibial and trabecular bone loss in adult OVX rats (23, 26). In postmenopausal women receiving estrogen-replacement therapy, exercise training did not produce any changes in blood lipid profiles (12), whereas an increase in peak exercise hemodynamics has been reported (10). However, the interactions between exercise training and estrogen replacement on glucose metabolism have never been considered. The second aim of the present study was to determine the effects of an 8-wk period of estrogen replacement in untrained and trained OVX rats on glucose tolerance and glucose-stimulated insulin response. The estrogen replacement, ensured by the estrogens released from a pellet placed subcutaneously, resulted in plasma levels of 17β-estradiol that can be considered as hyperestrogenic.

METHODS

Animal care. Female Sprague-Dawley strain rats (n = 59; Charles River, St-Constant, PQ, Canada), 6 wk old, weighing 180 ± 12 g, were divided randomly into four groups: sham-operated, ovariectomized (OVX), ovariectomized estradiol-treated (OVXE2), and ovariectomized estradiol treated and treadmill run (OVXE2T). Animals were housed in individual cages, with a 12-h light-dark cycle (lights on at 0500), in a temperature-controlled environment (22°C ± 3°C). Animals were allowed free access to food and water. Food intake was measured daily and expressed as g/day. Body weight was measured weekly, and body fat and lean body mass were determined at the end of the experiment using a body composition analyzer (Inbody 100, Bodystat, Isle of Man, U.K.).

Training. Exercise training was performed in a treadmill (eight wheels) at speeds adjusted to the rats’ treadmill speed. Rats were trained for 60 min/day, 3 days/week for 4 wk, starting at a speed that was 20% lower than the speed at which the animals ran to exhaustion. The speeds were progressively increased to 80% of the maximal speed determinated in each rat. One group of rats was trained on the treadmill from the age of 30 days (young rats), whereas the other was trained from the age of 90 days (adult rats). The mean daily running distance was 16.8 ± 1.5 km.

Blood and tissue collection. At the end of the experiment, all rats were fasted for 12 h and then intravenously injected with a potassium chloride solution (0.49 M) until they showed a complete loss of righting reflex. Blood samples were obtained from the abdominal aorta and stored at −80°C until analysis. The left ventricle of the heart was removed, and its wet weight was recorded. The left thigh muscle (gastrocnemius) and the whole brain were removed, and their wet weights were measured. Tissues were stored at −80°C until analysis.

Plasma glucose levels were determined using the glucose oxidase method (Glucose Oxidase Reagent, Sigma, St Louis, MO). Plasma insulin levels were measured with a rat insulinlic assay (Linco, St Charles, MO). Plasma estradiol levels were measured with a rat estradiol assay (Linco) and validated against the estradiol assay described by Simoni et al. (25). Plasma leptin levels were determined with an ELISA kit (Linco). Plasma insoluble collagen (collagen content) levels were determined with a collagen C assay (Bioclin, L’Hôpital, France).

Histological examination. The left tibia and the lumbar vertebrae were fixed in 10% formalin. The tibiae were embedded in paraffin wax, sectioned, and stained with picrosirius red. The vertebrae were dehydrated and embedded in methylmethacrylate. Sections were cut at 4 μm and stained with toluidine blue. Histomorphometric analyses were performed on images taken with a Nikon microscope (n = 12 animals per group).

Statistical analysis. All data are expressed as means ± SE. The statistical significance of differences among groups was analyzed with one-way ANOVA followed by a Tukey-Kramer’s post hoc test. A value of P < 0.05 was considered statistically significant.


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180–200 g were housed individually and fed pellet rat chow and tap water ad libitum after they were received in our laboratory. The 12:12-h light-dark cycle started at 6:00 AM, and the room temperature was maintained at 20–23°C. All rats were treated similarly in terms of daily manipulations. The experiments described in this report were conducted according to the directives of the Canadian Council on Animal Care.

**Surgery.** Three days after their arrival, rats were divided into three groups (n = 19–20 rats/group); the first two groups underwent OVX with and without estrogen replacement and the third group was sham operated (Sham). OVX was conducted according to the technique described by Robertson et al. (21). Animals were first anesthetized with pentobarbital sodium (40 mg/kg ip) and then injected intramuscularly with antibiotics (penicillin G procaine; 40,000 U/kg) and subcutaneously with an analgesic (buprenorphine: Temgesic, 0.025 mg/kg). Next, a small incision (5 mm) was made through the skin and the muscle back walls in parallel with the animal’s body line. The ovaries were then located, and a silk thread (5-0) was tightly tied around the oviduct, including the ovarian blood vessels. The oviduct was sectioned, and the ovary was removed, taking good care in leaving the knot intact. The skin and the muscle wall were then sutured with a silk thread (4-0). Sham rats were treated in a similar way, but the ovaries and oviduct were only manipulated. For OVX rats with estrogen replacement (OVXE2), a small 17β-estradiol pellet (catalog no. SE-121; Innovative Research of America, Sarasota, FL) was placed subcutaneously between the shoulder blades. The estrogen released from the pellet was 1.5 mg of 17β-estradiol (0.025 mg/day) with a biodegradable carrier binder efficient for 60 days. A placebo 60-day pellet containing the binding carrier only was used for all other rats (catalog no. SC-111). The estrous cycle, the completeness of OVX, and the efficiency of estrogen replacement were verified by daily administration of a vaginal smear test to all animals within the first 4 wk after surgery. All OVX rats were in the proestrus stage, which is characterized by the unique presence of nucleated epithelial cells stained with a 0.1% Giemsa solution and observed under light microscopy (×100).

**Groups and training protocol.** Two days after surgery, the three groups (Sham, OVX, and OVXE2) were subdivided into trained and sedentary subgroups. All together, there were six groups of experimental animals: three groups of trained rats and three groups of sedentary rats. Exercise training consisted of continuous running on a motor-driven rodent treadmill (Quinton Instruments, Seattle, WA) five times a week for 8 wk. Rats were progressively run from 15 min/day at 15 m/min, 0% slope, up to 60 min/day at 26 m/min, 10% slope, for the last 4 wk. All rats were monitored several times a week for their body weight and food intake, which was accurately measured over 2 consecutive days. Around 30 g of food pellets were accurately weighed and transferred in the food box where the dropping food debris and leftover pellets were collected and subtracted from the original food weight the following afternoon.

**Intravenous glucose tolerance test.** Two days after the last training session, all rats were submitted to an intravenous glucose tolerance test (ivGTT) while in an overnight-fasted state (~15 h). The experiments were run between 08:00 AM and 12:00 PM. The ivGTT was conducted according to a modified technique of Bongbele et al. (5). On the morning of the test, all rats were anesthetized with pentobarbital sodium (40 mg/kg ip) and shaved on both the frontal and back portions of the neck. A venous catheter was inserted in the right jugular vein (PE50) and was kept patent with a solution of sterile saline heparin (5 U/ml) for the entire duration of the test. An intramuscular injection of penicillin (penicillin G procaine; 40,000 U/kg) was also done to prevent possible infection. A period of 15 min was allocated between the completion of the surgery and the beginning of the ivGTT to standardize the effects of the surgical stress. The ivGTT consisted of injection of an intravenous glucose bolus of 0.5 g/kg of 50% dextrose solution, administered over a period of 10 s at time 0 min. The catheter was rinsed four to six times with the animal’s blood to remove any residual glucose. Blood samples (0.5 ml) were collected before (~5 and 0 min) and 2.5, 5, 15, 25, 35, and 60 min after the administration of the glucose load and used for subsequent glucose and insulin analyses. From each blood sampling, red blood cells were resuspended in sterile saline heparin solution (5 U/ml) and intravenously reinjected into the rat. At the end of the ivGTT, the jugular catheter was tunneled, closed, and attached behind the neck of the animal. All rats were allowed to recover and returned to their cages.

Five days after the ivGTT, all rats were weighed and their catheters were connected with an extension (PE50) used to reanesthetize them (20 mg/kg iv pentobarbital sodium) after an overnight fast. After complete anesthesia, the abdominal cavity was rapidly opened and ~5 ml of blood were quickly collected via the abdominal vena cava (~45 s). Immediately thereafter, removal and electronic weighing (Mettler AE 100) of the uterus were done to assess both the OVX and estrogen replacement effects of the present investigation.

**Analytic methods.** Peripheral blood was collected into 5-ml syringes with EDTA (7%). Blood was centrifuged, and the plasma was used for estradiol glucose and insulin determinations. Plasma samples were stored at −78°C until analyses were performed. Plasma glucose concentrations were determined with the use of a glucose-lactate analyzer (Yellow Springs Instruments 2300, Yellow Springs, OH). Plasma insulin concentrations were determined by a commercially available radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA, distributed by Medicorp, Montreal, PQ). Plasma 17β-estradiol concentrations were determined with a radioimmunoassay kit available from Buhlmann Laboratories. All data are reported as means ± SE. Statistical analyses were performed by a two-way ANOVA for nonrepeated measures with exercise training and insulin levels as main effects. The area under the curve of each rat was computed using a trapezoidal model to facilitate the analysis of the kinetic recording of the body weight, food intake, and both insulin and glucose plasma levels during the ivGTT. A Tukey’s post hoc test was used in the event of a significant (P < 0.05) F ratio.

**RESULTS**

There were no significant (P > 0.05) interactions of the main effects (exercise training and estrogen levels) for any of the reported variables. OVX resulted in a significant (P < 0.01) reduction of 17β-estradiol levels (Fig. 1A). However, 17β-estradiol levels in OVX rats were still ~75% of those measured in Sham. Estrogen replacement with the subcutaneous pellets resulted in 17β-estradiol levels that were approximately two-times higher than that in the Sham groups (P < 0.01). For this reason, the estrogen-replacement rats will be considered from now on as hyperestrogenic rats. OVX significantly (P < 0.01) reduced the uterus weight in OVX rats compared with results shown in both Sham and OVXE2 groups (Fig. 1B). Plasma insulin levels were significantly (P < 0.01) higher in OVX and...
OVXE2 rats compared with Sham (Fig. 1C), whereas plasma glucose levels were significantly ($P < 0.01$) lower in OVXE2 rats compared with the two other groups (Fig. 1D). There were no significant ($P > 0.05$) main effects of exercise training on any of the above-mentioned variables in any of the groups (Fig. 1).

As expected, OVX rats, compared with Sham, had a significantly ($P < 0.01$) higher body weight gain during the experimental 8-wk period (Fig. 2). In contrast, the 17β-estradiol replacement therapy resulted in a reduction of the normal weight gain compared with that shown in the Sham group. Food intake was significantly ($P < 0.01$) higher in OVX rats than in Sham, whereas food intake in OVXE2 rats was intermediate between OVX and Sham rats (Fig. 3). There were no significant ($P > 0.05$) main effects of training on either body weight or food intake in any of the groups (Figs. 2–3).

Plasma glucose and insulin responses during the ivGTT are presented in Figs. 4 and 5. OVX did not change the overall blood glucose response to the ivGTT, compared with that shown in Sham. A small but significant ($P < 0.02$) lower plasma glucose response during the ivGTT was, however, found in the OVXE2 rats compared with the OVX rats (Fig. 4). No main effects of training were found on the ivGTT blood glucose response. There were no significant ($P > 0.05$) main effects of the estrogen level on GSIR (Fig. 5). However, a main effect ($P < 0.001$) of training on GSIR was found in all three groups (Fig. 5).

**DISCUSSION**

OVX resulted in a significant reduction in circulating plasma 17β-estradiol levels compared with that shown in the Sham group. Estradiol levels in OVX rats were, however, still ~75% of normal values at the end of the experiment. This must not be interpreted as an unsuccessful surgery. Vaginal smear tests performed during the experiment do indicate that the OVX and the estrogen replacement were successful. In addition, the important reduction in the uterus weight, measured at the end of the experiment, clearly shows that OVX rats were, most likely, hypoestrogenic during a good part of the experiment. Substantial levels of plasma estradiol in OVX rats have been observed in previous long-
duration experiments (22) and suggest that other organs, such as the adrenal glands, might have taken over secretion of estradiol. Estrogen replacement used in the present study resulted in higher than normal levels of 17β-estradiol and higher than normal uterus weight at the end of the experiment. Higher than normal levels of estradiol have also been observed in other experiments using estrogen injections (13, 22).

As previously reported (20, 25), OVX resulted in a significant weight gain most likely brought about by an increase in food intake. The estrogen replacement in the present study reduced the body weight gain below the values observed in the Sham group, whereas food intake was reduced midway between Sham and OVX animals. In the present study, the first observation was that training did not reduce weight gain or food intake in the OVX as well as in the hyperestrogenic and the intact rats. This is in agreement with previous findings made on intact female rats (17) as well as on OVX rats (9, 20). This does not mean that body composition was not changed by training. It does mean, however, that any other changes related to training cannot be attributed to a change in body weight.

Results of the present ivGTT do not indicate the presence of a state of insulin resistance following OVX. However, an indication that an insulin-resistant condition was progressively developing is the observation of basal insulin levels that were higher in OVX rats than in the Sham groups (Fig. 1C). Impaired glucose tolerance, decreased glycogen deposition in various insulin-sensitive tissues, and increased gluconeogenesis have been observed in OVX rodents (2, 3, 13). Although it is well documented that a deficiency in circulating levels of estrogen influences insulin action, the exact mechanisms involved in these changes are not well defined (11). It is well known that an increase in body weight resulting in obesity is characterized by a state of insulin resistance. It is not clear, however, if the deterioration of insulin action in OVX rats is related to the increases in food intake and body weight or to the lower estrogen levels per se or to a combination of both. One of the purposes of the present experiment was to determine if insulin-stimulated glucose uptake in estrogen-deficient rats adapts differently to an endurance training program. Data from the present study indicate that exercise training did result in a reduction
of insulin response, as measured by ivGTT, in OVX rats similar to that observed in the Sham groups. This response indicates that exercise training does improve overall insulin sensitivity in OVX rats. The improvement in GSIR following training in OVX rats as well as in Sham was independent of any changes in body weight. It has not been excluded, however, that the exercise effects on insulin sensitivity in Sham and OVX rats might be related to a reduction in fat gain, even though body weight was not affected (20). Overall, the present data suggest that a decrease in ovarian steroid hormones does not impair overall metabolic adaptations related to an improvement in GSIR, which is similarly to what was reported in skeletal muscle GLUT-4 glucose transporter content (11).

The measurement of basal plasma 17β-estradiol at the end of the experiment indicates a higher than normal level of plasma estradiol in OVX rats with estrogen replacement. It is likely that the rate of diffusion of estrogen from the pellets used in the present experiment resulted in above-normal plasma estrogen levels. Because the plasma estrogen levels were not measured during the experiment, it is not possible to know precisely whether estrogen levels were higher than normal throughout the experiment. However, the higher than normal uterus weight in OVXE2 rats suggests that these rats in this condition were hyperestrogenic during most of the experiment. As previously reported (14, 20), estrogen replacement resulted in a significant reduction in body weight gain in OVX rats, indicating that the low-estrogen level in OVX rats was responsible for the increase in body weight. Accordingly, estrogen replacement also resulted in an attenuation of the increased food intake observed in OVX rats. The mechanism by which estrogen exerts major influences on eating behavior and body weight regulation is still unclear. Recent data on this matter reveal an involvement of the ovarian steroid hormones in the regulation of the ob gene (27) and an interaction of ovarian hormones with the presence of corticosterone (8).

Estrogen replacement in the present study had some effects on glucose metabolism, as basal glycemia (Fig. 1D) and plasma glucose levels during the ivGTT were significantly lower than what was observed in OVX rats. It has been reported in previous studies that estrogen replacement may reverse deterioration of in-
sulin action brought about by ovarian hormone deficiency (2, 3, 13). Estrogen replacement has also been reported to improve glucose metabolism and plasma lipids in postmenopausal women with non-insulin-dependent diabetes mellitus (1). The present results on glucose metabolism in OVXE2 rats suggest that hyperestrogenic rats were more insulin sensitive than the OVX rats. The effects of estrogen replacement or hyperestrogenemia on improvements of glucose homeostasis might be related to the decrease in food intake and body weight in this condition. Chronic moderate reduction in energy intake (4–20 days) has been reported to result in increased insulin sensitivity and improved glucose homeostasis in rats (6, 7). It is not excluded, however, that estrogen levels per se or in combination with the reduction in food intake and body weight contribute to the improvements in glucose homeostasis observed in OVXE2 rats. The second purpose of the present study was to determine how exercise training and estrogen hormone replacement interact to improve glucose tolerance and GSIR. The results of the present study indicate that estrogen replacement had no effect on the improvement of GSIR in trained animals. In previous studies (12, 16), it was reported that exercise training and estrogen therapy provided no additional benefit to the effects of estrogen alone on the lipid profiles of postmenopausal women. The present results indicate that estrogen replacement to the level and the duration employed in the present study, similar to an estrogen deficiency, does not impair or increase the metabolic adaptations responsible for an improvement of GSIR following endurance exercise training.

In summary, exercise training in OVX rats with or without estrogen replacement results in an improvement of the GSIR similar to that observed in intact female rats. Our findings suggest that ovarian hormones of the levels and duration employed in the present study do not influence the adaptations of GSIR elicited by a regular endurance-type exercise. On a practical point of view, the present data suggest that pre- and postmenopausal women can benefit from exercising regularly, counteracting some possibly developing metabolic abnormalities such as insulin resistance.

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