Placental and vascular adaptations to exercise training before and during pregnancy in the rat

Jeffrey S. Gilbert,1 Christopher T. Banek,1 Ashley J. Bauer,1 Anne Gingery,1 and Hans C. Dreyer2

1Department of Physiology and Pharmacology, University of Minnesota Medical School, Duluth, Minnesota; and 2Department of Human Physiology, University of Oregon, Eugene, Oregon

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THERE IS INCREASING INTEREST in the potential role of exercise during pregnancy in mitigating the effects of placental growth factor (PlGF) on vessels that have exercised (40). Moreover, stimulating pro-angiogenic factors such as PlGF and vascular endothelial growth factor (VEGF) may be beneficial in mitigating the development of hypertensive disorders of pregnancy such as preeclampsia. In addition, other circulating factors such as leptin have been shown to have important pro-angiogenic role, and the effect of exercise during pregnancy on circulating leptin concentrations in the rat has not been studied.

Whereas recent work suggests that regular exercise during uncomplicated pregnancies may improve vascular endothelial function (37), the factors responsible for this improvement in vascular function remain unclear. In addition, exercise is known to stimulate the expression of a number of cytokines that are thought to initiate the development of pregnancy-induced hypertension and preeclampsia.

Considering that recent reports suggest exercise may be useful as a therapeutic agent in the treatment or prevention of hypertensive disorders of pregnancy, the exact mechanisms by which exercise exerts these influences remain nebulous. To this end we sought to test the hypothesis that voluntary exercise before and during pregnancy in the rat would stimulate cytoprotective molecules such as heat shock proteins (HSP) and antioxidants in the placenta, decrease anti-angiogenic factors such as heat shock proteins (HSP) and antioxidant enzymes in myocytes (1, 38, 43). Taken together, these findings suggest exercise may mitigate the effects of placental ischemia that is thought to initiate the development of pregnancy-induced hypertension and preeclampsia.

Methods and apparatus

Animals. Studies were performed in primiparous Sprague-Dawley rats purchased from Charles River (Portage, MI). Animals were housed in a temperature-controlled room (23°C) with a 12:12 light:dark cycle. All experimental procedures performed in this study were in accordance with National Institutes of Health guidelines for use and care of animals. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Minnesota. Rats were randomly assigned to either the exercise group (n = 8) or the nonexercise control group (n = 10).

Standard rodent wire activity wheels were fitted with a cyclocomputer, which included a magnet that was attached to the wheel, and an electronic magnetic sensor. The device measured distance calculated from the number of wheel rotations and the wheel circumference manually programmed into the unit. Voluntary wheel running was chosen for this study to minimize potentially deleterious effects that
have been previously reported with treadmill running in Sprague-Dawley rats (30).

After 6 wk of exercise on the activity wheels or no exercise in the control group, breeding pairs were placed in a wire bottom cage and the presence of two or more vaginal plugs was observed to confirm mating. That day was designated as gestation day 1. Females were returned to individual cages with exercise wheels (Pregnant + Exercise) or without wheels (Pregnant) until the morning of gestation day 19.

Measurement of mean arterial pressure in chronically instrumented conscious rats. Animals were instrumented on day 17 of gestation, and arterial pressure was determined in both groups of conscious rats at day 19 of gestation using an indwelling arterial catheter placed in the carotid artery as described previously (12, 14, 18). Heart rate data were obtained from the blood pressure recordings using Powerlab software as reported previously (12).

Conceptus measurements and tissue collection. After the measurement of blood pressure, the dams were placed under isoflurane anesthesia, and a midline ventral incision was made to isolate the abdominal aorta for plasma and serum collection as reported previously (14, 18). Pups and placentas were excised, and the location within the uterine horn was recorded and weighed. Tissues were snap frozen in liquid nitrogen and stored at −80°C until further analyses were performed. Placental efficiency was defined as the fetal weight per placental weight as described previously by our group and others (12, 39).

Determination of fetal position. Uterine position was determined by numbering the fetus closest to the ovary as number one and then numbered in ascending order toward the cervix as we have previously reported (13, 30).

Plasma/serum/assays. Blood was collected for subsequent assays into Corvac sterile serum separator tubes (Sherwood Davis, St. Louis, MO), and plasma was collected into BD Vacutainer EDTA-containing tubes. Circulating VEGF (R & D Systems Minneapolis, MN), leptin, (Millipore; Billerica, MA), and sFlt-1 concentrations were measured using commercial ELISA kits available from R&D systems (Quantikine; Minneapolis, MN) according to the manufacturer’s directions as described previously (4, 14). Trolox-equivalent antioxidant capacity of the plasma was assessed with a total antioxidant assay (Cayman Chemical) as described previously (14, 18).

Protein extraction and quantitation. As described previously (14), total soluble protein was extracted from whole placentas and whole gastrocnemius muscle in radioimmunoprecipitation assay (RIPA) lysis buffer containing phenylmethylsulfonylfluoride (PMSF) in dimethyl sulfoxide (DMSO), sodium orthovanadate, and a protease inhibitor cocktail (Santa Cruz Biotechnology). Total soluble cellular protein concentration was determined using the bichinchoninic acid (BCA) method (Pierce Biotechnology) as described previously (13, 18).

Western blot. Protein (50 µg) was separated by electrophoresis on 4–20% sodium dodecyl sulfate (SDS) polyacrylamide separating gels (Novex, Invitrogen), then transferred to nitrocellulose membranes (Bio-Rad), and Ponceau stained to assure even transfer across each gel. The images of the Ponceau-stained membranes were digitized using a flatbed scanner (Hewlet-Packard).

The membranes were then incubated 1 h in casein blocking solution (Bio-Rad). Membranes were incubated in blocking solution containing commercially available antibodies (from Abcam unless noted otherwise) for HSP 27 (ab12351, 1:5,000), HSP 60 (ab46798, 1:5,000), HSP 90 (ab1429, 1:5,000), ATP synthase (ATP5A, ab147481, 1:250), and PGC-1 (Santa Cruz, sc-13067, 1:1,000) overnight at 4°C. Membranes were washed and incubated 1 h with the appropriate horseradish peroxidase-conjugated secondary antibodies (1:10,000–1:20,000, Cell Signaling) and incubated in chemiluminescent substrate (West Femto, Pierce). The immunoreactive bands were digitized using an Alpha-Innotech digital imaging system. All digitized images were quantified using Un-Scan-It gel 6.1 software (Silk Scientific, Orem, UT). Specificity of primary antibodies (negative controls) was evaluated by imaging membranes with the primary antibody omitted.

Vascular wire myography experiments. Wire myography experiments were performed as previously described (4, 16). Briefly, mesenteric vessels were dissected between the second and third branch points. Mounting and vessel normalization were performed using the LabChart 6.0 software and according to the DMT Multi Wire Myography System User Manual (model 610 M) 2006. After normalization, vessels were allowed to rest for 20 min and then preconstricted with the thromboxane mimetic U-46619 (10–4 M). After vessel preconstriction with U-46619, endothelial function was assessed by the addition of acetylcholine (ACH) and sodium nitroprusside (SNP) to the vessel bath to achieve cumulative dose-response curves (10–9 to 10–4 M) as previously reported (4, 16).

Statistical analysis and calculations. All data are presented as means ± SE. Western immunoblot data are presented as the ratio of target protein to β-actin. Conceptus data were calculated as mean per pregnancy. Comparisons between two groups were made with a t-test for independent samples, and a Welch’s correction for unequal variances was applied when indicated. Uterine position data was evaluated by two-way ANOVA. Nonlinear regression to compare best-fit curves was used to analyze cumulative dose-response curve data. Statistical significance was accepted when P < 0.05. Statistical calculations were made with GraphPad Prism (GraphPad Software, San Diego, CA).

RESULTS

Exercise data. Figure 1 illustrates that the daily distance run by the rats was consistent between the prepregnancy period and the first 2 wk of gestation (Fig. 1). The distance run by the pregnant rats decreased (P < 0.05) in the final week of pregnancy. To demonstrate that the amount of voluntary wheel running done by the rats in this study was sufficient to induce metabolic training adaptations, we chose to measure ATP synthase and PGC1-α expression in skeletal muscle. Figure 2 illustrates that ATP synthase (Fig. 2A) and PGC1-α (Fig. 2B) expression was increased (P < 0.05) in the gastrocnemius muscle of the exercise-trained rats compared with the nonexercised rats.

Maternal and conceptus morphometrics. Maternal heart weight was not different between the exercise and nonexercise groups (data not shown). Fetal weight (2.59 ± 0.22 vs. 2.55 ± 0.06 g) was not altered in the exercise-trained compared with...
in the exercise-trained group compared with the nonexercised rats. No difference was observed between the groups with respect to endothelium independent relaxation to SNP (data not shown).

Circulating angiogenic factor and antioxidant status, and placental antioxidant status. Exercise training increased maternal plasma antioxidant status [3.5 ± 0.3 vs. 1.5 ± 0.3 mM of trolox equivalents (teq); P < 0.05] in pregnant rats compared with nonpregnant rats. Placental antioxidant status (17.5 ± 0.7 vs. 17.1 ± 0.8 mM teq) was not different between the exercise-trained and nontrained rats. A TBARS assay indicated that levels of malondialdehyde (22.3 ± 1.6 vs. 22.4 ± 1.1 mmol/l), an index of lipid peroxidation, was not different between the placentas of exercise-trained and nontrained rats.

Angiogenic balance (sFlt-1:VEGF) was improved (i.e., decreased) by exercise training in pregnancy. Figure 8 shows that circulating VEGF was increased in the exercise-trained rats compared with the nonexercised group (Fig. 8A) and that this effect of exercise on plasma VEGF was pregnancy specific. Figure 8B shows that the sFlt-1/VEGF ratio decreased in the exercise-trained rats. Leptin and sFlt-1 (data not shown) were unchanged by exercise training and were not different between any of the groups.

Effects of chronic exercise training on HSP expression in skeletal muscle and placenta. Figure 4 illustrates that chronic exercise training before and during pregnancy increased placental expression of HSP 27 relative to β-actin (3.7 ± 0.36 vs. 2.2 ± 0.4; P < 0.05) on day 19 of pregnancy. In addition, HSP 60 (2.2 ± 0.7 vs. 0.5 ± 0.1; P < 0.05; Fig. 5) and HSP 90 (0.32 ± 0.10 vs. 0.11 ± 0.02; P < 0.05; Fig. 6) were increased in exercised compared with nonexercised rats.

In contrast to the placenta, chronic exercise training before and during pregnancy did not result in any changes in skeletal muscle expression of HSP 27, 60, or 90 (data not shown).

Blood pressure and heart rate during late gestation. Mean arterial pressure (101 ± 3 vs. 99 ± 3 mmHg) and heart rate (461 ± 13 vs. 433 ± 10 beats/min) on day 19 of pregnancy were not different between the exercise and nonexercise groups.

Vascular function. The diameters of the mesenteric vessels dissected for this study were similar between the exercise and nonexercise groups (184 ± 26 vs. 178 ± 18 μm). Figure 7 illustrates that mesenteric vessel relaxation to ACh was greater in the exercise-trained pregnant rats compared with pregnant rats in the nonexercise group. Furthermore, the log EC50 (−7.48 ± 0.08 vs. −7.19 ± 0.12 M ACh; P < 0.05) was lower the nonexercise group. In contrast, placental (Fig. 3A; P < 0.05) weight was decreased in the exercise-trained compared with the nonexercised rats. Furthermore, placental efficiency was increased (Fig. 3B; P < 0.05) in the exercise-trained rats. There was no difference in the number of resorptions (1.3 ± 0.5 vs. 1.0 ± 1.0) between the exercise and nonexercise groups. There was no effect of exercise on fetal or placental size at different locations in the uterus.

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DISCUSSION

The present study reveals several interesting and novel findings regarding maternal and placental adaptations to chronic exercise training before and during pregnancy. Foremost, we observed increased expression of several cytoprotective molecules in the placenta, increased circulating antioxidant factors, increased VEGF, and augmented endothelium-mediated vascular relaxation in late pregnancy. Furthermore, we also report that the effects of exercise training on circulating levels of VEGF were pregnancy specific as no changes were observed in age- and exercise-matched nonpregnant virgin rats. Second, we report that exercise training increases expression of several HSP (HSP 27, 60, and 90) in the rat placenta. Thus these findings identify potential pathways by which exercise may have beneficial and perhaps protective effects during pregnancy.

We chose to employ a voluntary wheel running paradigm to avoid the stress that has previously been associated with treadmill running as an activity intervention (31). Since the amount of exercise was not strictly controlled, we first determined whether there was evidence of metabolic adaptations in the skeletal muscle of the animals given free access to an activity wheel. Indeed we found that the amount of wheel running by the rats before and during pregnancy was sufficient to induce an increase in gastrocnemius ATP synthase and PGC1-α expression.

In the current study we found that there was no difference in maternal weight gain during the last week of pregnancy nor were there changes in cardiac mass in the exercise compared

![Fig. 4. Effects of chronic exercise training on heat shock protein (HSP) 27 expression in placenta. Exercise training before and during pregnancy increased placental expression of HSP27 relative to β-actin. Data are expressed as means ± SE, *P < 0.05.](image)

![Fig. 5. Effects of chronic exercise training on HSP 60 expression in placenta. Chronic exercise training before and during pregnancy increased placental expression of HSP 60 relative to β-actin. Data are expressed as means ± SE, *P < 0.05.](image)

![Fig. 6. Effects of chronic exercise training on HSP 90 expression in placenta. Chronic exercise training before and during pregnancy increased placental expression of HSP 90 relative to β-actin. Data are expressed as means ± SE, *P < 0.05.](image)

![Fig. 7. Endothelial function in late gestation. Wire myography revealed that vessel relaxation to acetylcholine (ACh) (after preconstruction with U41669) was increased in mesenteric vessels of the exercise-trained pregnant rats compared with pregnant rats in the nonexercise group. Data are expressed as mean percent relaxation to each dose of ACh ± SE, *P < 0.05.](image)
with nonexercise groups. We presently report that the spontaneous wheel activity by the rats did not affect fetal growth by day 19 of pregnancy. We also report that placental weight was decreased, but that placental efficiency (fetal weight:placental weight) was increased by exercise training before and during pregnancy in the rat. Previous studies have reported a wide range of effects of exercise before and during pregnancy on conceptus weight in both women and rats. Prior work has largely been equivocal with findings at term gestation reporting reduced (8, 20, 22, 33), increased (7), and no change in conceptus weights (21, 25, 42). The underlying differences between these observations remain unclear, but the wide variation in the amounts and types of exercise are likely contributing factors. Nevertheless, our present finding showing an increased placental efficiency in the exercise-trained rats is consistent with our report that angiogenic balance is improved in these animals as well. Further studies are planned to determine whether indices of placenta function (e.g., transport of amino acids, vascular density) are improved by exercise training.

One shortcoming of previous studies evaluating the effects of exercise during pregnancy on fetal-placental outcomes is the lack of putative mechanistic insights from these investigations. Our current findings reveal that exercise induces increases in several cytoprotective molecules in the placenta and circulation that may provide some mechanistic insights regarding the previously reported positive effects of exercise on cardiovascular and placental function. We found that HSP 27, 60, and 90 were increased in the placenta of the exercise-trained rats in late gestation. A number of previous studies have reported that induction of a variety of HSPs has beneficial effects on cellular function in a variety of organs, including the placenta, heart, skeletal muscle, and vasculature (11, 17, 24, 35).

Small HSPs such as HSP 27 are reported to have a number of important cellular functions ranging from stabilization of protein folding and decreased oxidative stress and apoptotic signaling (32). Moreover, recent evidence suggests that HSP 27 may play a role in mediating stimulation of VEGF secretion by certain cell types (26). Similarly, larger HSPs such as HSP 90 have been reported to work in conjunction with endothelial nitric oxide synthase (eNOS) to facilitate increased NO production (34). Whereas there appear to be clear links between HSP 27 and HSP 90 and the maintenance of proper vascular function, the role of HSP remains unclear. Recent evidence suggests that HSP 60 may play an important role in stimulating prosurvival pathways via activation of the inhibitor of κB kinase (IkB, or IKK) that phosphorylates the IkB protein in two amino-terminal serine residues, leading to the consequent liberation of NF-κB proteins (6). Thus the stimulation of HSPs by exercise during pregnancy may represent an important preconditioning pathway that promotes placental and cardiovascular function during gestation.

Gestational exercise has been shown to have some beneficial effects in mothers with a predisposition to pregnancy-induced hypertension in a clinical setting (44), and recent studies in both women and mice have provided data supporting the hypothesis that exercise training before and during pregnancy may represent a potential preventative or treatment modality (9, 40). Our findings that VEGF is increased and sFlt-1/VEGF ratio is decreased are in agreement with previous reports that exercise training before and during pregnancy improves the angiogenic profile of pregnant women and rats. In addition, we found that this effect of exercise was pregnancy specific, suggesting that the placenta plays an important role in this effect of exercise on circulating angiogenic balance. This is in agreement with previous studies that have reported exercise increases skeletal muscle (10, 19) and circulating VEGF acutely after exercise bouts but that exercise training does not increase basal serum concentrations of VEGF (27). Despite these intriguing results, it remains unclear if the placenta is the source of the increased circulating VEGF or if factors secreted by the placenta may act on skeletal muscle to enhance VEGF levels in pregnancy. Further studies are ongoing in our laboratory to evaluate these possibilities.

We also report that endothelial function is improved in late gestation of pregnant rats of our exercise group. This set of data provides further evidence that exercise training may be of considerable benefit to women at risk for developing hypertensive disorders of pregnancy. While it is important to currently recognize that exercise is presently contraindicated during preeclampsia, this position is largely founded on putative risks as opposed to empirical data reporting deleterious effects of exercise on preeclamptic women and their offspring. Nevertheless, further studies are clearly indicated to determine if these concerns are warranted.
Perspectives and Significance

While the design of the present study precludes us from extending these findings to the well being of the offspring, it is apparent from the present work that there was no decrease in fetal survival or fetal size due to the voluntary exercise by the rat dams. Nevertheless, the present findings suggest that physical activity before and during pregnancy stimulates several molecular pathways that may yield benefits with respect to placental and/or vascular function in the gravida. These include a promotion of angiogenic balance (increased VEGF), which may contribute to the augmented vascular endothelial relaxation that could potentially mitigate effects of antiangiogenic factors (such as sFlt-1) in conditions such as preeclampsia. Moreover, the stimulation of placental HSP expression may help maintain adequate prosurvival signaling for the maintenance of placental and vascular function during periods of placental ischemia or insufficiency that are associated with fetal growth restriction and preeclampsia. While the present data identify potential mechanisms by which exercise may have benefits for women at risk of developing hypertensive disorders of pregnancy, it must be recognized that further studies are required to evaluate the safety and efficacy of this possibility in models of hypertension during pregnancy.

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Current address for A. Gingery: Dept. of Biochemistry & Molecular Biology, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905 (gingery.anna@mayo.edu).

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.S.G. conception and design of research; J.S.G., C.T.B., and A.J.B. prepared figures; J.S.G. drafted manuscript; J.S.G., C.T.B., A.J.B., and H.C.D. approved final version of manuscript.

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