Exercise Training Attenuates Placental Ischemia-Induced Hypertension and Angiogenic Imbalance in the Rat

Jeffrey S. Gilbert, Christopher T. Banek, Ashley J. Bauer, Anne Gingery, Karen Needham

Abstract—An imbalance between proangiogenic (vascular endothelial growth factor) and antiangiogenic (soluble fms-like tyrosine kinase 1) factors plays an important role in hypertension associated with reduced uteroplacental perfusion (RUPP). Exercise has been shown to stimulate proangiogenic factors, such as vascular endothelial growth factor, in both the pregnant and nonpregnant state; thus, we hypothesized that exercise training would attenuate both angiogenic imbalance and hypertension attributed to RUPP. Four groups of animals were studied, RUPP and normal pregnant controls and normal pregnant and RUPP+exercise training. Exercise training attenuated RUPP-induced hypertension (P<0.05), decreased soluble fms-like tyrosine kinase 1 (P<0.05), increased VEGF (P<0.05), and elevated the soluble fms-like tyrosine kinase 1:vascular endothelial growth factor ratio. The positive effects of exercise on angiogenic balance in the RUPP rats were confirmed by restoration (P<0.05) of the RUPP-induced decrease in endothelial tube formation in human umbilical vascular endothelial cells treated with serum from each of the experimental groups. Placental prolyl hydroxylase 1 was increased (P<0.05) in RUPP+exercise training rats. Decreased trolox equivalent antioxidant capacity in the placenta, amniotic fluid, and kidney of the RUPP rats was reversed by exercise. RUPP-induced increase in renal thiobarbituric acid reactive species was attenuated by exercise. The present data show that exercise training before and during pregnancy attenuates placental ischemia-induced hypertension, angiogenic imbalance, and oxidative stress in the RUPP rat and reveals that increased prolyl hydroxylase 1 is associated with increased soluble fms-like tyrosine kinase 1, thus revealing several potential pathways for exercise training to mitigate the effects of placental ischemia-induced hypertension. Lastly, the present study demonstrates that exercise training may be a useful approach to attenuate the development of placental ischemia-induced hypertension during pregnancy. (Hypertension. 2012;60:1545-1551.)

Key Words: preeclampsia ■ pregnancy ■ vascular endothelial growth factor A ■ blood pressure

Hypertensive disorders during pregnancy, such as preeclampsia (PE), occur in ≈5% to 8% of pregnancies. Because of its prevalence, PE is a substantial source of maternal and fetal morbidity and mortality, as well as premature delivery. Despite recent advances that have identified and begun to optimize the use of several new biomarkers of PE, current treatment options remain limited.1 Although the exact mechanisms of the pathogenesis of PE remain unclear, recent studies continue to support the hypothesis that a poorly perfused, ischemic placenta leading to an imbalance in hypoxia-inducible factor (HIF) regulated angiogenic factors (eg, soluble fms-like tyrosine kinase 1 [sFlt-1], vascular endothelial growth factor [VEGF], and placental growth factor), along with increased renal and placental oxidative stress2 and widespread endothelial dysfunction, is central to the development of the disease.3,4

Previous work from clinical studies has shown that exercise during pregnancy promotes placental growth4 and angiogenic balance.5 In addition, there is growing interest in the possibility that exercise during pregnancy may hold therapeutic potential to mitigate the effects of placental insufficiency or the angiogenic imbalance associated with PE. Several reports suggest that exercise during pregnancy may positively influence fetal growth and later developmental milestones.6,7 Recent evidence suggests that 10 weeks of exercise during pregnancy lowers diastolic blood pressure in women with a predisposition to pregnancy-induced hypertension10 and decreases their cardiovascular risk profile.7 Other studies have shown recently that exercise during pregnancy may promote a proangiogenic state by increasing placental growth factor in pregnant women.6 Similar findings have been reported recently by our group and others in studies using rodent models.11,12 Thus, it has been proposed that stimulating proangiogenic factors, such as placental growth factor and VEGF, may be beneficial in mitigating the development of hypertensive disorders of pregnancy, such as PE.

In addition to the recent findings regarding exercise and angiogenic factors, exercise training has long been observed to stimulate the expression of a number of cytoprotective molecules, such as antioxidant enzymes,13–16 and data suggest...
that oxygen-sensing molecules, such as HIF-1α and prolyl hydroxylases (PHDs) are also influenced by exercise.\textsuperscript{17} Taken together, these findings suggest that exercise may mitigate the effects of placental ischemia that are thought to initiate the development of pregnancy-induced hypertension and PE. Despite the potential for positive outcomes associated with exercise during pregnancy, it has remained contraindicated in pregnancies complicated with high blood pressure.\textsuperscript{18} To this end, we sought to test the hypothesis that voluntary exercise before and during pregnancy in the rat would decrease blood pressure and oxidative stress in the placenta and kidney, as well as restore angiogenic balance in pregnant rats with hypertension that develops from chronic reductions in uterine perfusion pressure (RUPP).

Methods

Animals

Studies were performed in age-matched female Sprague-Dawley rats purchased from Charles River (Portage, MI) at 3 weeks of age. Animals were housed in a temperature-controlled room (23°C) with a 12:12 light:dark cycle. Rats were assigned to one of the following experimental groups, normal pregnant (NP, n=8), NP-exercise (NP+Ex, n=6), RUPP (n=10), or RUPP+exercise (RUPP+Ex, n=7). All of the experimental procedures executed in this study were in accordance with National Institutes of Health guidelines for the care and use of animals. All of the protocols were approved by the institutional animal care and use committee at the University of Minnesota.

Exercise Wheels, Quantification of Exercise, and Breeding

Standard rodent wire activity wheels were fitted with a cyclocomputer, and running distance was measured weekly, as described previously.\textsuperscript{12} Voluntary wheel running was chosen for this study to minimize potentially deleterious effects that have been reported previously with treadmill running in Sprague-Dawley rats.\textsuperscript{19} Furthermore, we have reported previously that this period of exercise is sufficient to stimulate increases in mitochondrial markers of exercise adaptation, peroxisome proliferator-activated receptor gamma coactivator-α and ATP synthase.\textsuperscript{12} After 6 weeks 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 ≥2 vaginal plugs was observed to confirm mating. That day was designated as gestation day 1. Females were returned to individual cages with exercise wheels or without wheels until the morning of gestation day 19.

RUPP Procedure

The RUPP procedure is a well-established model for studying the link between placental ischemia and hypertension in the pregnant rat and has been described in detail previously.\textsuperscript{20,21} In brief, silver clips were placed on the lower abdominal aorta (0.203-mm ID) above the iliac bifurcation and also on branches (0.100-mm ID) of both the right and left ovarian arteries supplying the uterus on day 14 of pregnancy (term, 21). Normal pregnant rats all underwent a sham surgery, which included the midline incision and suture.

Measurement of Mean Arterial Pressure in Chronically Instrumented Conscious Rats

Animals were instrumented on day 17 of gestation, and arterial pressure was determined in conscious rats at day 19 of gestation using an indwelling arterial catheter placed in the carotid artery, as described previously.\textsuperscript{22,23}

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.\textsuperscript{23} Amniotic fluid was collected by aspirating samples into a syringe with a 27-gauge needle. Pups and placentas were excised blotted dry and weighed. Tissues were snap frozen in liquid nitrogen and stored at −80°C until further analyses were performed.

Plasma, Serum, and Assays

Blood was collected for subsequent assays into Corvac sterile serum separator tubes (Sherwood Davis, St Louis, MO) and plasma into BD Vacutainer EDTA containing tubes. Circulating VEGF and sFlt-1 (R&D Systems, Minneapolis, MN) concentrations were measured using commercial ELISA kits available from R&D Systems (Quantikine) according to the manufacturer’s directions, as described previously.\textsuperscript{21} Oxidative stress was assessed by measuring total antioxidant capacity and thiobarbituric acid reactive species assay, which measures malondialdehyde. Total antioxidant capacity was assessed in amniotic fluid, placenta, and renal tissue by measuring Trolox equivalent antioxidant capacity assay kit (Cayman Chemical Company, Ann Arbor, MI) according to the manufacturer’s directions, as described previously.\textsuperscript{22,24} In addition, malondialdehyde was measured in kidney tissue by using a thiobarbituric acid reactive species assay (Cayman Chemical Company) according to the manufacturer’s directions, as described previously.\textsuperscript{22}

Protein Extraction and Quantitation

As described previously,\textsuperscript{12,23} total soluble protein was extracted from whole placentas and whole kidneys in radioimmunoprecipitation assay lysis buffer containing phenylmethanesulphonyl fluoride in dimethyl sulfoxide, sodium orthovanadate, and a protease inhibitor mixture (Santa Cruz Biotechnology, Inc). Total soluble cellular protein concentration was determined using the bicinchoninic acid method (Pierce Biotechnology).

Western Blot

Western immunoblots were performed in placenta, skeletal muscle, and renal tissue, as described previously, using 50 μg of total protein per lane separated on a Bis-Tris polyacrylamide gel (Invitrogen) and transferred to nitrocellulose membrane.\textsuperscript{12,24} Blots were probed for superoxide dismutase 2 (Abcam, ab13533; 1:5000), prolyl hydroxylase 1 (PHD1; Abcam, 86980; 1:1000), β-actin (Abcam, ab8226; 1:5000), and α-tubulin (Cell Signaling; 9099, 1:1000).

Endothelial Tube Formation Assay

Angiogenic balance was also assessed in the serum of pregnant rats in vitro, as we have published previously.\textsuperscript{25} Briefly, Phenol-red free growth factor reduced Matrigel (BD Biosciences, San Jose, CA) and plated at 50,000 cells/mL of serum-less medium. The cells were then treated with a 5% serum from the respective rat treatment groups and incubated for 8 hours. Tube formations per frame were assessed at ×40 optical zoom with a digital inverted compound microscope and ImageJ analysis software (National Institutes of Health, Bethesda, MD) by ≥2 individual investigators who were blinded to the identity of the experimental groups. Values from each observer were averaged to obtain final counts.

Statistical Analysis and Calculations

All data are presented as mean±SEM, and statistical significance was accepted when \( P < 0.05 \). sFlt-1/VEGF and sFlt-1 data were square root transformed before statistical analysis. Conceptus data were calculated as mean per pregnancy. Comparisons between experimental groups were 2-way ANOVA, and post hoc tests were used when indicated.
Results

Exercise Amount
There was no difference in the amount of wheel running per week before pregnancy in the animals from the NP and RUPP groups (30.4 versus 30.0 km/wk). Likewise, there was no difference in the amount of wheel running per week during pregnancy between the NP and RUPP rats (4.5 versus 4.8 km/wk).

Blood Pressure
Figure 1 illustrates that RUPP-induced hypertension was decreased (P<0.05) by wheel running before and during pregnancy but was still greater than the NP control rats. Wheel running did not alter blood pressure in the NP+Ex group.

Conceptus Morphometrics
Figure 2A illustrates that exercise before and during pregnancy had no effect on fetal weight in either the RUPP or NP groups. Figure 2B demonstrates that placental weight, which was decreased by RUPP when compared with NP rats, was further decreased by exercise in the RUPP group. Figure 2C shows that placental efficiency was not altered between the NP and RUPP rats in the present cohort but was increased in the RUPP+Ex group.

Angiogenic and Endocrine Factors
Figure 3A shows that circulating free levels of VEGF that were decreased (P<0.05) in RUPP were increased by wheel running. Likewise, Figure 3B illustrates that the sFlt-1/VEGF ratio, which was increased by RUPP, was decreased in wheel running before and during pregnancy. In addition, circulating levels of sFlt-1 were increased (P<0.05) in the RUPP rats when compared with the NP, NP+Ex, and RUPP+Ex rats (1300.0±345.8 vs 334.8±104.8 vs 110.0±22.0 pg/mL). Figure 4 shows that VEGF expression was increased (P<0.05) in the skeletal muscle of the RUPP+Ex rats compared with the NP and RUPP groups. Figure 5 illustrates that PHD1 was increased (P<0.05) in the placentas of the RUPP+Ex rats compared with the NP, RUPP, and NP+Ex groups.

Endothelial Cell Function
Figure 6 shows that endothelial cell tubule formation is decreased (P<0.05) with the addition of RUPP serum to the cells. The serum from RUPP+Ex rats showed more tubule formation when compared with the RUPP rats.

Oxidative Stress Data
Figure 7A shows that placental antioxidant capacity was decreased (P<0.05) in the RUPP group, and this was attenuated by exercise in the RUPP group. Exercise did not augment total antioxidant status of placental tissue in the NP rats. Figure 7B shows that amniotic fluid antioxidant status was decreased (P<0.05) in RUPP rats, and this was reversed by exercise training. Moreover, Figure 7C shows that exercise training increased (P<0.05) antioxidant capacity in the kidneys of the NP rats. Figure 7D shows that renal malondialdehyde was increased (P<0.05) in the RUPP kidneys and that this was attenuated by exercise in the RUPP group. Figure 8 illustrates that superoxide dismutase 2 was increased (P<0.05) in both exercise groups compared with the respective nonexercise groups. Western blot
analysis indicated renal superoxide dismutase 2 was not altered in any of the groups (data not shown).

**Discussion**

The present study reveals several interesting and novel findings regarding the effects of exercise before and during pregnancy on several aspects of maternal and fetal physiology in a robust model of hypertension during PE. Foremost, we observed that the placental ischemia-induced hypertension in this model was attenuated by exercise, and this was accompanied by restoration of angiogenic balance (ie, increased free VEGF, decreased sFlt-1, and increased endothelial cell tube formation in vitro). We also report that exercise training significantly increased VEGF expression in skeletal muscle of RUPP+Ex rats and increased PHD1 expression in the placenta from RUPP+Ex rats. Furthermore, exercise resulted in reduced oxidative stress in the RUPP hypertensive rat by reversing decreased antioxidant capacity in the placental, kidney, and amniotic fluid and mitigating the RUPP-induced increase in renal thiobarbituric acid reactive species. Thus, these findings demonstrate that exercise has several beneficial effects in rats with placental ischemia-induced hypertension.

We chose to use a voluntary wheel running paradigm that has been shown previously to elicit metabolic adaptations in the skeletal muscle (ie, increased gastrocnemius ATP synthase and peroxisome proliferator-activated receptor-γ coactivator-α expression) of the animals given free access to an activity wheel for the same amount of time as the rats in the current study and to avoid the stress that has been associated previously with treadmill running as an activity intervention. In the current study, we also found that the spontaneous wheel activity was not different in rats with or without a RUPP clip. Thus, despite reductions in blood flow to lower extremities because of the clips, there was sufficient autoregulation of blood flow to meet the metabolic demands of voluntary running.

Blood pressure is one of the chief concerns in PE, and our present data show that exercise before and during pregnancy is effective at reducing the extent of hypertension in the RUPP rat. This is in agreement with a recent study that used a transgenic approach in mice to study hypertension during pregnancy. Despite the significant reduction in blood pressure from the RUPP to the RUPP+Ex groups in this study, blood pressure was still elevated compared with the NP group. Although in some hypertensive settings this may not be desirable, in the...
are unsure of the significance of this observation at the present time. Taken together, there did not seem to be any significant fetal distress in the RUPP rats that exercised before and during pregnancy. In contrast, placental weight was decreased in the RUPP rats that exercised. In contrast to previous reports, we did not observe any decrease in placental efficiency between the RUPP and NP groups in the present cohort, but we did observe an increase in placental efficiency because of exercise in the RUPP rats. This observation is consistent with the restoration of angiogenic balance in the animals and the proangiogenic effects that we observed when human umbilical vascular endothelial cells were cultured with sera from the groups in this study and may suggest the presence of increased angiogenesis in the placentas of these animals. It should be noted that the placental weight change is difficult to evaluate in isolation, because this could be interpreted as either increased placental efficiency or as a sign of placental stress.

Thus, further studies are needed to evaluate placental function and vascularity, as well as possible effects on the fetus and offspring health, especially considering recent reports suggesting that maternal exercise during pregnancy may attenuate the fetal programming effects of a low-protein diet in rats.

The importance of angiogenic factors in the pathophysiology of hypertension during PE has become increasingly clear in recent years, and this has been confirmed in animal models. Thus, a primary focus of this study was to determine whether exercise training before and during pregnancy had any effect on angiogenic balance in RUPP hypertension. To this end, we found that free plasma VEGF was increased and plasma sFlt-1 was decreased. Moreover, we found that the angiogenic potential of the serum from exercised RUPP rats was also restored to normal pregnant levels. These studies are in agreement with previous work in women, rats, and mice that suggest that exercise during pregnancy has beneficial effects on angiogenic balance. Taken together, these data provide evidence that the mechanism by which exercise

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*Figure 6. Endothelial cell tube formation assay. Endothelial cell tubule formation was decreased with the addition of reduced uterine perfusion pressure (RUPP) serum to the cells when compared with cells treated with serum from normal pregnant (NP) rats. This effect was not observed when cells were treated with serum from RUPP rats that exercised. Data are expressed as means±SEM. Statistical significance for comparisons that are different by post hoc testing are indicated by lines above the bars. *P<0.05.*

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*Figure 7. Total antioxidant capacity in placenta, amniotic fluid, kidney, and thiobarbituric acid reactive species (TBARS; malondialdehyde) in kidney. A, Placental antioxidant capacity was decreased (P<0.05) in the reduced uterine perfusion pressure (RUPP) group, and this was attenuated by exercise in the RUPP rats. Exercise did not augment total antioxidant status of placental tissue in the normal pregnant (NP) rats. B, shows that amniotic fluid antioxidant status was decreased (P<0.05) in RUPP vs NP, and this was reversed by exercise training. C shows that renal antioxidant capacity was decreased (P<0.05) in the kidneys from RUPP vs NP rats and that this was attenuated by exercise in the RUPP group. Exercise alone did not augment total antioxidant status of renal tissue. D shows that renal malondialdehyde (MDA) was increased in the RUPP vs NP rats, and this was abrogated by exercise training. Data are expressed as means±SEM. Statistical significance for comparisons that are different by post hoc testing are indicated by lines above the bars. *P<0.05.*
HIF–mediated pathway to stimulate VEGF. This observation coactivator–receptor–γ shown that stimulation of peroxisome proliferator-activated receptors, as well as heme oxygenase 1. Moreover, recent work has reported previously in RUPP rats. In the present study, renal oxidative stress in the RUPP rat was improved by chronic exercise training before and during pregnancy. We also observed that the dependence on ischemia for pathway activation in the placenta has been reported recently by George et al. Although our observations that exercise training before and during pregnancy lowers sFlt-1 and increases VEGF levels are exciting, the mechanism by which exercise influences these angiogenic factors remains unclear. We showed recently that exercise training in rats increases circulating VEGF in pregnant but not in nonpregnant rats, which suggested that the placenta is a likely source of the increased VEGF. In this study we show that VEGF was increased in the gastrocnemius muscle of the rats in the RUPP+Ex compared with the RUPP group, suggesting that there may be a nonplacenta source of VEGF in these studies.

We have shown previously that RUPP hypertension and angiogenic imbalance are associated with increased placental HIF-1α expression, and the HIF pathway along with associated regulatory proteins, such as PHDs, have been suggested as putative targets to control factors governing angiogenic functions. We found that PHD1 was increased in the placentas of the RUPP+Ex rats compared with the NP, RUPP, and NP+Ex groups. Although we only found that PHD1 was increased in the RUPP rats, the dependence on ischemia for pathway activation in the placenta has been reported recently by George et al. with respect to adenosine regulation of angiogenic factors, as well as heme oxygenase 1. Moreover, recent work has shown that stimulation of peroxisome proliferator-activated receptor-γ coactivator-α in skeletal muscle represents a non-HIF–mediated pathway to stimulate VEGF. This observation remains correlative at this point but reveals a potential regulatory pathway by which regular physical activity may influence angiogenic balance in the setting of placental ischemia.

We also observed that exercise before and during pregnancy improved the antioxidant status of RUPP rats and attenuated the oxidative stress that we observed presently and others have reported previously in RUPP rats. In the present study, renal oxidative stress in the RUPP rat was improved by chronic exercise training before and during pregnancy. We report that antioxidant capacity was increased by exercise, and renal thiobarbituric acid reactive species, which are increased in the RUPP kidneys, was attenuated by exercise. Previous work has shown that antioxidant treatment lowers blood pressure in the RUPP model; thus, the decrease in renal oxidative stress may contribute to an improvement in renal excretory function and the reduction in blood pressure observed in the present study. We also show that placental and amniotic fluid antioxidant capacity was decreased in the RUPP group, and this was attenuated by exercise in the RUPP group. Excess reactive oxygen species in the intrauterine environment has been implicated in developmental programming, but whether a decrease of reactive oxygen species alters fetal development remains unclear. Nevertheless, this is an intriguing observation that deserves further attention in subsequent experiments.

Perspectives and Significance
Currently, PE is a disorder with very limited treatment options. The present data suggest that regular exercise before and during pregnancy reduces blood pressure in hypertensive pregnant rats and attenuates several sequelae of placental ischemia, such as angiogenic imbalance and oxidative stress. Although the design of the present study precluded 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 because of the voluntary exercise by the hypertensive rats.

Our findings also support the hypothesis that exercise training promotes angiogenic balance during pregnancy with placental ischemia. Furthermore, the observation that increased PHD1 was associated with decreased sFlt-1 suggests that disruption of HIF signaling may play a role. Although these observations are encouraging, it remains unclear whether regular exercise training before pregnancy is important to these observations. Considering that both pregnancy and exercise can be considerable physiological stressors, it seems likely that prepregnancy exercise status may play a role in whether exercise during pregnancy is effective and possibly safe as a preventive measure for increased blood pressure in pregnancy. Further studies are planned in our laboratory to evaluate these possibilities.

Viewed in concert with previous studies indicating that physical activity lowers risk for PE and other hypertensive disorders of pregnancy, the data from our study raise the possibility that exercise regimens may be an important way for women to mitigate the risk of PE. There are certainly questions that remain, such as when and how much exercise is required and whether exercise can safely be used as a therapeutic modality to mitigate effects of placental-ischemia–induced hypertension; nevertheless, we feel that these positive results are encouraging and further studies are warranted.

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Disclosures
None.

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**Novelty and Significance**

**What Is New?**

- This study shows that exercise training before and during pregnancy stimulates a proangiogenic state, reduces oxidative stress, and lowers blood pressure in a rat model of hypertension during PE that results from placental ischemia and mimics many of the features of the human condition. We also provide evidence that exercise training before and during a hypertensive pregnancy is associated with increased PHD1, a factor that may decrease HIF-mediated sFlt-1 signaling and did not result in any adverse fetal consequences in late gestation.

**What Is Relevant?**

- There are currently no treatments for PE other than close management of the expectant mother and delivery of the infant when needed. Despite the long-recognized benefits of exercise on cardiovascular health, exercise has been traditionally contraindicated during hypertensive pregnancies. This study provides important evidence indicating that exercise training before and during pregnancy has beneficial effects on maternal cardiovascular function.

**Summary**

Exercise before and during pregnancy may have beneficial effects on blood pressure and angiogenic balance in PE.
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