Exercise in Pregnancy Increases Placental Angiogenin without Changes in Oxidative or Endoplasmic Reticulum Stress

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ABSTRACT

HARDY, D. B., X. MU, K. S. MARCHIORI, and M. F. MOTTOLA. Exercise in Pregnancy Increases Placental Angiogenin without Changes in Oxidative or Endoplasmic Reticulum Stress. *Med. Sci. Sports Exerc.*, Vol. 53, No. 9, pp. 1846–1854, 2021. **Purpose:** Despite immense research highlighting maternal–fetal benefits of exercise during pregnancy, there remain concerns that exercise may undermine placental function. Although maternal exercise has demonstrated favorable aerobic conditioning responses in the mother, it is not known whether maternal exercise promotes increased angiogenesis in the placenta, perhaps at the expense of impaired endoplasmic reticulum (ER) homeostasis and/or oxidative stress. We investigated if a mild (30% heart rate reserve) and/or moderate (70% heart rate reserve) exercise regime in healthy pregnant women affected placental markers of angiogenesis, ER stress, and oxidative stress. We hypothesized that the improved aerobic conditioning of mothers who exercise is beneficial to enhance placental angiogenesis and normal maternal–fetal outcomes. **Methods:** Placental tissues were collected within 1 h of delivery from a convenience sample of 29 healthy mothers of full-term infants. Twenty-one women participated in routine exercise from midgestation (16–20 wk) until term of either mild or moderate intensity, whereas eight sedentary women served as controls. **Results:** No differences were identified between groups including gestational length, fetal–placental weight ratio, or APGAR scoring. All exercisers exhibited a significant 20-fold increase in the mRNA (as assessed by quantitative real-time polymerase chain reaction) and a 10-fold increase in the protein expression of angiogenin (e.g., ANG1) in the placenta. However, in both exercising groups, no increases in placental markers (i.e., HIF1α, VEGF), ER stress (i.e., spliced XBP1, ATF4, ATF6, CHOP, and BAX), or oxidative stress (i.e., SOD1, SOD2) were observed. **Conclusions:** Overall, our study suggests that mild- and moderate-intensity exercise increases angiogenesis but does not increase placental oxidative or ER stress in healthy pregnancies, bolstering support for routine exercise as a part of standard care in pregnant women. Future studies are warranted to investigate the potential benefits of exercise on ANG1 in pathological pregnancies. **Key Words:** MATERNAL EXERCISE, PLACENTA, ANGIOGENIN, ER HOMEOSTASIS, HYPOXIA, MATERNAL–FETAL

Despite recent evidence that exercise throughout pregnancy is efficacious for healthy maternal and fetal outcomes (1), maternal barriers to physical activity still exist because of the perception of exercise being unsafe for the mother and fetus (2). In fact, recent systematic reviews and meta-analyses have shown that prenatal exercise was not associated with miscarriage or perinatal mortality (3), preterm/prelabor rupture of membranes (4), neonatal complications, or adverse childhood outcomes (5). In addition, prenatal exercise did not adversely affect fetal heart rate or uteroplacental blood flow metrics (6) but did reduce the odds of macrosomia (>4.0 kg) (5).

Exercise has been linked to angiogenesis in many tissues of the body in nonpregnant individuals and seems to be more pronounced with aerobic exercise (7). Because the placenta is the conduit between maternal and fetal nutrient and oxygen/carbon dioxide exchange as it responds to the maternal environment, it seems reasonable that maternal exercise may play a role in adaptive placental responses to protect the fetus. We have previously

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shown that a walking program of 20 wk of low-intensity (30% heart rate reserve, or HRR) or moderate-intensity (70% HRR) increased cardiorespiratory responses in the mother suggesting an aerobic conditioning response with both intensities of exercise (8). This beneficial adaptive aerobic response may also affect placental physiology, specifically with respect to the expression of angiogenin 1 (ANG1) and angiopoetin-2 (ANGTP2), which are both involved in increasing blood vessel formation in the placenta (9–11). Given the links between aerobic exercise and angiogenesis, we first sought to examine if these markers of angiogenesis (e.g., ANG1 and ANGTP2) in the placenta were influenced by exercise in pregnancy and if so, does intensity of that activity produce different results with regard to placental responses.

Placental efficiency has been defined as grams of birth weight per gram of placenta and is associated with fetal growth (12). Although alterations in angiogenesis and vascular structure within the placenta may improve efficiency and function, rapid changes in hemodynamics could also result in endoplasmic reticulum (ER) stress (13,14). The ER is responsible for many cellular functions, including protein synthesis (15). ER stress is induced from an accumulation of misfolded and/or unfolded proteins in the ER lumen (15). To restore ER homeostasis, the uncoupled protein response (UPR) is activated through three signaling pathways: IRE1, ATF6, and PERK (16). The IRE1 pathway is involved in the upregulation of chaperone proteins to fold unfolded or misfolded proteins, mediated by increases in the expression of the transcription factor, spliced XBP-1 (15). The second pathway involves increases in ATF6 expression, which is further responsible for enhancing and assisting with protein folding (15). Finally, the PERK pathway (i.e., increases in ATF4) is involved in the attenuation of protein translation to reduce the amount of new proteins entering the ER lumen (15). However, if UPR fails to alleviate ER stress, downstream apoptotic pathways (i.e., bcl-2-like protein 4 (BAX)) are activated, mediated through C/EBP-homologous protein/Gadd153 (CHOP), which has been associated with compromised placental development and function *in vivo* (i.e., gestational diabetes, idiopathic intrauterine growth restriction (IUGR), preeclampsia) and *in vitro* (17–21). To date, it remains unknown if exercise in pregnancy leads to alterations in oxygenation, which could drive placental ER stress. Given the close proximity of the ER and mitochondria, integrated signaling occurs between both organelles whereby a dysregulated ER can also lead to greater oxidative stress (as indirectly measured by increases in the antioxidant superoxide dismustases, SOD1–1 and SOD2) (22,23), which are also attributed to placental insufficiency (i.e., IUGR, preeclampsia) (19,22,24,25). But to date, the effects of exercise in pregnancy on placental oxidative stress also remain elusive.

Our purpose is to investigate if a mild (30% HRR) and/or moderate (70% HRR) exercise regime in healthy pregnant women impacted placental markers of angiogenesis, ER stress, and oxidative stress compared with nonexercisers. We hypothesize that the improved aerobic conditioning of mothers who exercise in pregnancy (regardless of intensity, mild or moderate) is beneficial to enhanced placental angiogenesis and normal maternal–fetal outcomes compared with pregnant women who do not exercise. However, given the increased demands of exercise on the maternal system, which may include the placenta, we also predict that if angiogenesis is increased in the placenta, it may be attributed to increased ER and oxidative stress. Therefore, the objectives were to identify whether routine maternal exercise of two different intensities (mild or moderate) promotes placental angiogenesis (i.e., ANG1 and ANGTP2) (9–11) perhaps due to increased ER and oxidative stress.

METHODOLOGY

A convenience sample of pregnant women between 16 and 20 wk of gestation was recruited through referrals from medical professionals, posters, and advertisements in newspapers in London, Ontario, Canada. Before being enrolled in the study, women were medically prescreened (26) by their health care provider. Specific exclusion criteria included a maternal age greater than 40 yr, smoking, multiple pregnancy, presence of chronic diseases, or other contraindications to exercise (1). Ethics approval was obtained from the Human Research Ethics Board for Health Sciences at The University of Western Ontario, and written informed consent was obtained from participants.

After medical prescreening, at 16–20 wk of gestation, women completed a standardized ramp test to volitional fatigue on a cycle ergometer as previously described (27) to determine their current fitness level. After the exercise test, the women were randomized into an exercise program of either low-intensity (30% HRR) or moderate-intensity (70% HRR). Calculated target heart rates based on each individual fitness test were maintained throughout the exercise program that continued until delivery. Participants exercised on a stationary cycle ergometer or a stair climber, or attended a supervised exercise class. All participants wore heart rate monitors while exercising to maintain their appropriate target heart rate range, were required to exercise a minimum of three times per week, and attend all supervised exercise sessions. Duration of activity varied depending on exercise modality, but women were encouraged to meet pregnancy exercise guidelines of at least 150 $min \cdot wk^{-1}$ (1) at the appropriate group intensity. Heart rate was recorded while exercising, and all exercise sessions were recorded in an exercise log. A reference group of sedentary pregnant women were recruited in the third trimester of pregnancy (34–36 wk). These women completed an exercise questionnaire and confirmed that they did not participate in any structured exercise program.

Body mass index (BMI) was calculated from self-reported maternal prepregnancy body weight and measured height to the nearest centimeters using a stadiometer. Birth weight, APGAR (Appearance, Pulse, Grimace, Activity, and Respiration) scores taken at 1 and 5 min after birth (evaluates newborn health), placental weight, and gestational age at birth were recorded from medical records. Placental samples were harvested within 1 h of delivery. Each placenta was blotted and weighed, and tissue samples were taken from the fetal side

TABLE 1. Forward and reverse sequences for the primers used for quantitative real-time PCR.

Gene	Forward	Reverse	GenBank/Reference
Spliced XBP1	CCGCAGCAGGTGCAGG	GGGGCTTGGTATATATGTGG	NM_005080.3
XBP11	GGCCATGAGTTTTCTCTCGT	CGAATGAGTGAGCTGGAACA	NM_001079539.2
ATF6	TTGACATTTTTGGTCTTGTGG	GCAGAAGGGGAGACACATTT	XM_011509309.1
ATF4	CTATACCCAACAGGGCATCC	GTCCCTCCAACAACAGCAAG	NM_001675.4
CHOP	TGATGCTCCCAATTGTTCATG	TCGCCGAGCTCTGATTGAC	NM_001195053.1
VEGF	ATGACGAGGGCCTGGAGTGTG	CCTATGTGCTGGCCTTGGTGAG	NM_001287044.1
HIF1α	GCGGCGCGAACGACAAGAAA	TGGAAGTGGCAACTGATGAGCAAGC	NM_001530.4
ANG1	CATCATGAGGAGACGGGG	TCCAAGTGGACAGGTAAGCC	NM_001145.4
ANGTP2	CCCAGTCCACCTGAGGAACT	TGCTTTGGTCCGTTAAGTGAT	NM_001147.3
BAX	GGAGGAAGTCCAATGTCCAG	GGGTTGTCGCCCTTTTCTAC	NM_001291428.2
SOD1	CCACACCTTCACTGGTCCAT	CTAGCGAGTTATGGCGACG	NM_000454.5
SOD2	TGACCACCACCATTGAACTT	CGTCACCGAGGAGAAGTACC	NM_000636.4
GAPDH	AGGTCCACCACTGACACGTT	GCCTCAAGATCATCAGCAAT	NM_002046

after removal of the membrane immediately beside the umbilical cord. All fibrous tissue was removed from each sample and immediately clamp-frozen in liquid nitrogen. Clamps and containers to hold the sample were precooled in liquid nitrogen. Samples were stored at −80°C until analyses.

RNA extraction and real-time polymerase chain reaction. Total RNA was extracted from homogenized placenta samples using TRIzol reagent (Invitrogen). Chloroform (Sigma-Aldrich) was added to the solution, and then centrifuged at 12,500 rpm. Supernatant was transferred to a fresh tube with an equal volume of isopropanol (Sigma-Aldrich) and centrifuged again at 12,500 rpm. Total RNA was then collected from the pellet and dissolved in DEPC-treated water. Deoxyribonuclease I, Amplification Grade (Invitrogen), was added to the RNA to digest contaminating single- and double-stranded DNA. Four micrograms of RNA was reverse transcribed to cDNA using random hexamers and Superscript II Reverse Transcriptase (Invitrogen). Primer sets directed against gene targets of interest including markers of placental angiogenesis (i.e., ANG1, ANGT2), hypoxia (i.e., vascular endothelial growth factor (VEGF), hypoxia-inducible factor 1α (HIF1α)), ER stress (i.e., ATF4, ATF6, spliced Xbp-1, CHOP, BAX), and oxidative stress (i.e., SOD1, SOD2) were designed through National Center for Biotechnology Information's primer designing tool and generated via Invitrogen Custom DNA Oligos (Table 1). Quantitative analysis of mRNA expression was performed via real-time polymerase chain reaction (RT-PCR) using fluorescent nucleic acid dye SsoFast EvaGreen supermix (BioRad) and BioRad CFX384 Real Time System. The cycling conditions were 95°C for 10 min, followed by 43 cycles of 95°C for 15 s and 60°C for 30 s and 72°C for 30 s. The cycle threshold was set so that exponential increases in amplification were approximately level between all samples. Relative fold changes were calculated using the comparative cycle times (Ct) method, normalizing all values to GAPDH, which was selected as housekeeping gene. GAPDH was determined as a suitable housekeeping gene using algorithms from GeNorm, Normfinder, BestKeeper, and the comparative ΔCt method to place it as the most stable housekeeping gene from those tested (e.g., β-actin and 18S ribosomal RNA) (28–31). Given all primer sets had equal priming efficiency, the ΔCt values for each primer set were calibrated to the average of all control Ct values, and the relative abundance of each primer set compared with calibrator was determined by the formula $2^{\Delta\Delta Ct}$, in which ΔΔCt was the normalized value.

Protein extraction and Western blot. Placenta samples were homogenized in RIPA buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P40, 0.25% $C_{24}H_{39}NaO_4$, supplemented with 20 mM NaF, 40 mM Na-pyrophosphate, 40 mM Na_3VO_4, 200 mM β-glycerophosphate disodium salt hydrate, and protease inhibitor cocktail (Roche)). The solution was sonicated at 30% amplitude for 5 s total, 1 s per pulse. It was then mixed in a rotator for 10 min at 4°C and centrifuged at 300*g* for 15 min at 4°C. The supernatant was collected and centrifuged at 16,000*g* for 20 min at 4°C. The resulting supernatant was collected as the total cellular protein fraction and quantified by colorimetric DC protein assay (BioRad). Loading samples were prepared with NuPAGE LDS Sample Buffer (4×; Invitrogen), NuPAGE Reducing Agent (10×; Invitrogen), and deionized water, and heated at 70°C for 10 min to denature the proteins. Proteins (20 μg per well) were separated by size via gel electrophoresis in gradient polyacrylamide gels (Novex) and transferred onto polyvinylidene difluoride membrane (Millipore). Membranes were blocked in 1× Tris-buffered saline–Tween 20 buffer with 5% nonfat milk (blocking solution), and then probed using primary antibodies against ANG1 (catalog no. sc-74528; Santa Cruz Biotechnology, dilution 1:500) and β-actin (catalog no. A5441; Sigma-Aldrich, dilution 1:50,000), all diluted in the blocking solution. Secondary antimouse secondary antibodies (catalog no. 715-001-003;

TABLE 2. Participant demographics.

	Sedentary (*n* = 8)	All Active (*n* = 21)	30% HRR (*n* = 6)	70% HRR (*n* = 15)
BMI, kg·m^{-2}				
16–20 wk	N/A	25.4 ± 3.3	26.2 ± 3.0	25.1 ± 3.5
Gestational age, wk				
Initial visit	34.9.0 ± 0.6	17.1 ± 1.6	16.5 ± 1.8	17.4 ± 1.4
At delivery	40.5 ± 0.5	39.7 ± 1.6	40.1 ± 1.1	39.5 ± 1.7
Birth weight, g	3683.4 ± 159.5	3392.1 ± 462.6	3560.2 ± 343.8	3324.8 ± 496.5
Placental weight, g	616.3 ± 119.9	636.2 ± 91.6	606.7 ± 68.5	648.0 ± 99.0
Fetal–placental weight ratio	6.2 ± 1.0	5.4 ± 0.8	5.9 ± 0.5	5.2 ± 0.8
APGAR scores				
At 1 min	8.9 ± 0.6	8.1 ± 1.8	8.7 ± 1.9	7.8 ± 1.9
At 5 min	9.5 ± 0.8	9.2 ± 0.5	9.2 ± 0.4	9.2 ± 0.6

N/A, not applicable because the sedentary women were recruited between 34 and 36 wk of gestation.

Jackson ImmunoResearch Laboratories, dilution 1:10,000) were used to detect the species-specific portion of the primary antibody, all diluted in the blocking solution. Immunoreactive bands were visualized using SuperSignal West Dura Chemiluminescent Substrate (Thermo Scientific) and analyzed using BioRad Image Lab™ Software, and band intensities of ANG1 proteins were normalized to that of β-actin as previously published (32).

Statistical analyses. All statistical analyses were performed using GraphPad Prism 8 software. Descriptive statistics were reported as mean ± SD. All other results were expressed as means of normalized values ± SEM. The significance of the differences ($P < 0.05$) between normalized mean values of sedentary ($n = 8$) and total exercise groups ($n = 21$) was evaluated using an unpaired Student's *t*-test. If significant, exercise groups were stratified by 30% and 70% intensities, and a one-way ANOVA was performed followed by Tukey's posttest. Cohen's *d* tests were also performed to determine effect size; small, 0.20; medium, 0.50; and large, 0.80. If Cohen's *d* tests showed a large effect size, data from the exercise groups were then also stratified.

RESULTS

Effects of exercise in pregnancy on maternal–fetal outcomes. A convenience sample of 28 women were recruited for the exercise component of the study. We were not able to collect placental samples for seven women. Thirteen women were recruited for the sedentary reference group, and we were unable to collect placental samples from five women. Only data from those women with placental samples are presented here (Table 2). The average target exercise heart rate was 118 ± 8 bpm in the mild-intensity exercise group and 140 ± 7 bpm in the moderate-intensity exercise group. All

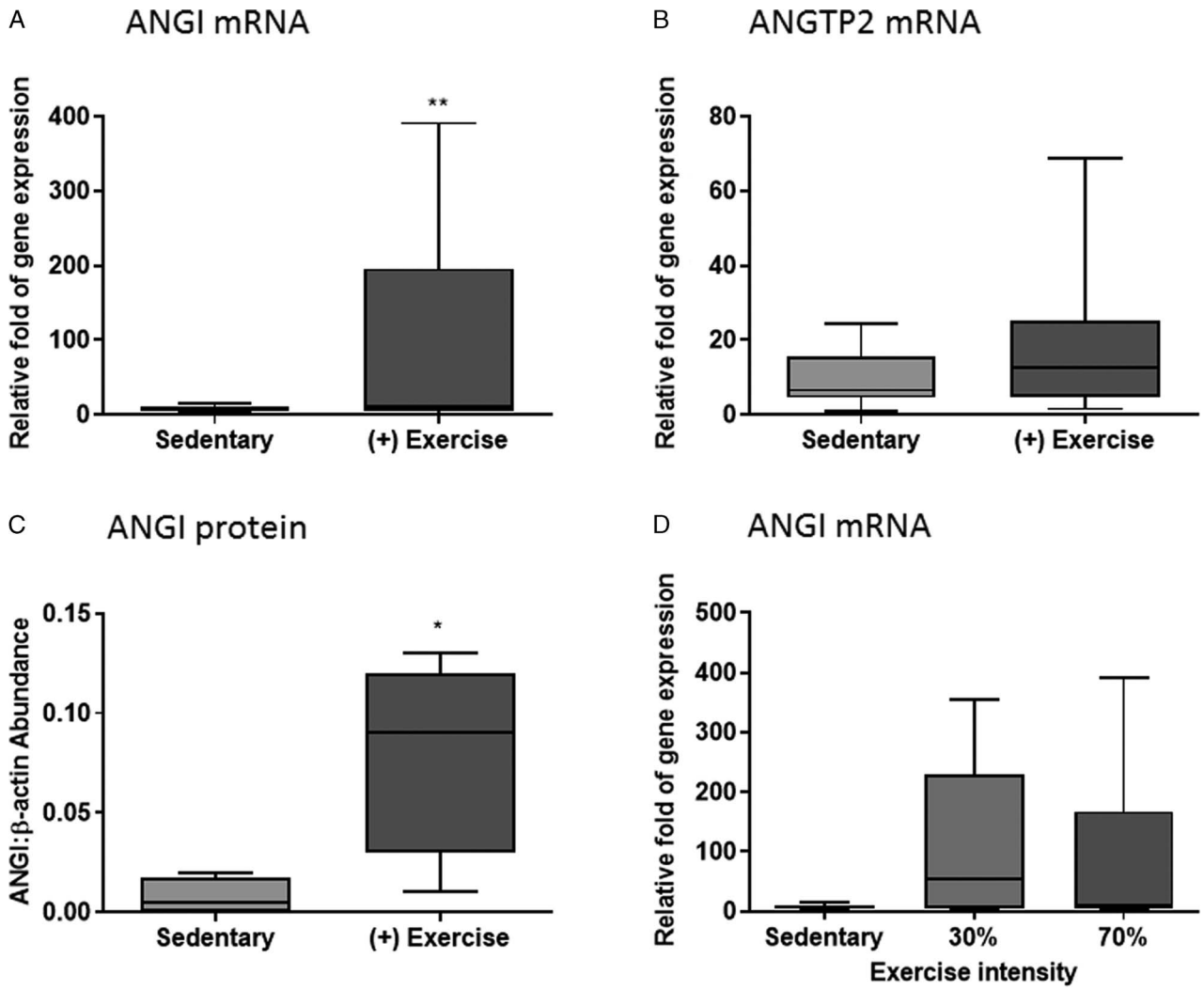

FIGURE 1—Exercise in pregnancy increases ANG1 expression in the human placenta. Relative expression of ANG1 mRNA (A), ANGTP2 mRNA (B), ANG1 protein (C), and ANG1 mRNA (D) between sedentary and exercising groups. To assess steady-state mRNA, real-time qPCR was used after total RNA was extracted from placentae and reverse-transcribed to cDNA and normalized to GAPDH. For protein analysis, ANG1 protein bands were detected by anti-ANG1 antibody via Western blot and normalized to β-actin. All values were expressed as mean ± SEM. Significant differences between treatment groups were determined by Student's *t*-test ($P < 0.05$). For panel D, differences between stratified exercise groups were assessed by a one-way ANOVA followed by Tukey's posttest ($P < 0.05$).

exercising women were able to meet the guideline of at least 150 min·wk^{-1}. Adherence to the exercise program was ≥70% in both exercise groups. BMI and gestational age (initial or at delivery) were not different among all groups examined (sedentary, all active, mild, or moderate exercise; Table 2). Moreover, exercise in pregnancy (i.e., mild, moderate, or both) had no effect on birth weight, placental weight, fetal–placental weight ratio, and APGAR scores (Table 2).

Effects of exercise in pregnancy on markers of placental angiogenesis. To address if exercise in pregnancy affects angiogenesis in the human placenta, we used quantitative RT-PCR (qRT-PCR) to assess the steady-state levels of ANG1 and ANGTP2(9–11). qRT-PCR revealed a significant 20-fold increase in the expression of ANG1 mRNA in the exercise group compared with the sedentary group (Fig. 1A, $P < 0.01$), but no change in ANGTP2 mRNA (Fig. 1B) at birth. When the protein levels of ANG1 were assessed, the exercise group exhibited a significant 10-fold increase in ANG1 protein compared with the sedentary group (Fig. 1C. $P < 0.05$). When the exercise group was stratified by 30% and 70% intensities, one-way ANOVA revealed no differences among these groups (Fig. 1D); however, Cohen's *d* test revealed a large effect size for sedentary versus 30% intensity (1.17) and sedentary versus 70% intensity (1.3), suggesting greater sample size could lead to a significant interaction in both exercise groups.

Effects of exercise in pregnancy on markers of placental hypoxia. Given placental pathology is often associated with augmented hypoxia in the placenta, we next explored if exercise in pregnancy contributed to elevations in the expression of VEGF and/or HIF1α, two known markers of placental hypoxia (10,33,34). Exercise in pregnancy did not alter the steady-state mRNA expression of VEGF mRNA (Fig. 2A) or HIF1α mRNA (Fig. 2B) at birth. Although there were no significant differences in these groups, when broken down to 30% and 70% exercise intensities (Fig. 2C), Cohen's *d* test revealed a large positive effect size for sedentary versus 70% intensity (1.07) with respect to VEGF mRNA (e.g., increased VEGF mRNA in the 70% intensity group), suggesting a greater sample size could lead to a significant interaction in the moderate exercise groups.

Effects of exercise in pregnancy on markers of placental ER stress and oxidative stress. As alterations in nutrient status and oxygenation can lead to placental ER stress, involved in the etiology of gestational diabetes, IUGR, and preeclampsia, we next investigated if mild or moderate exercise influenced ER stress by measuring known markers of each branch of the UPR (i.e., spliced XBP1, ATF4, ATF6). qRT-PCR revealed no significant changes in the steady-state mRNA expression of spliced XBP1 (Fig. 3A), ATF4 (Fig. 3B), or ATF6 (Fig. 3C). Moreover, given prolonged, unresolved ER stress can lead to apoptosis and cellular death, we also investigated the expression of CHOP and BAX, implicated in ER stress-induced apoptosis (15,16). Exercise in pregnancy had no effect on the overall placental expression of CHOP (Fig. 3D) or BAX (Fig. 3E) at birth. Knowing the close approximation of the ER and mitochondria and given impaired mitochondrial function results in oxidative stress and pathological placental outcomes (i.e., fetal growth restriction, preeclampsia) (24,25,35,36), we next measured the steady-state mRNA expression of the antioxidants, superoxide dismutase-1 (SOD1) and SOD2. Exercise in pregnancy had no major effect on the overall expression of SOD2 mRNA (Fig. 3G), but it did decrease the expression or SOD1 mRNA (Fig. 3F). When the exercise group was stratified by 30% and 70% intensities, one-way ANOVA revealed 30% or 70% exercise intensity groups both had significant decreases in SOD1 mRNA (sedentary vs 30%, $P < 0.05$; sedentary vs 70% intensity, $P < 0.05$, as determined by the Tukey *post hoc* test) compared with the sedentary group (Fig. 3H).

DISCUSSION

In the current study, we had hypothesized that, because of improved aerobic conditioning, mothers who exercised in pregnancy would exhibit increased placental angiogenesis and normal maternal–fetal outcomes. Moreover, given maternal exercise results in increased oxygen and glucose utilization (12) of the maternal system that potentially affects the placenta, we predicted that this may also result in increased placental ER and oxidative stress. Our results indicated that

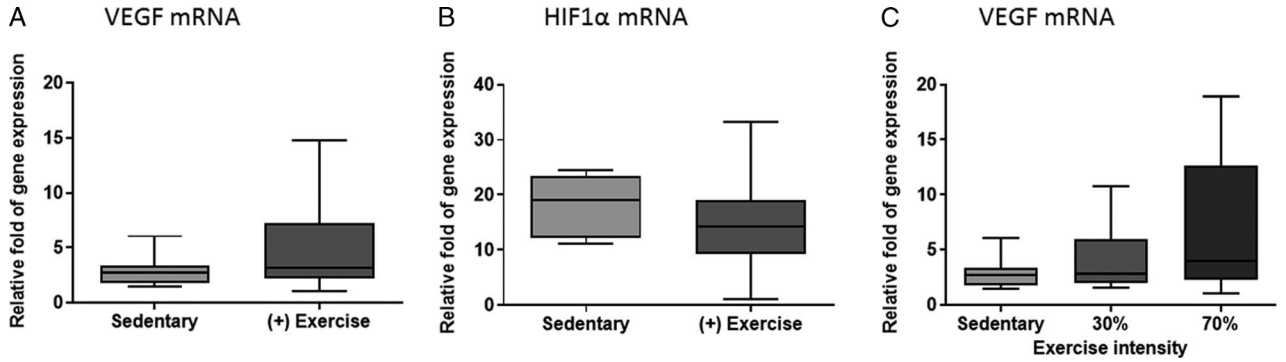

FIGURE 2—Exercise in pregnancy does not influence markers of hypoxia in the human placenta. Relative expression of VEGF mRNA (A), HIF-1α mRNA (B), and VEGF mRNA (C) between sedentary and exercising groups were assessed by real-time qPCR. Total RNA was extracted from placentae and reverse-transcribed to cDNA and normalized to GAPDH. All values were expressed as mean ± SEM. Significant differences between treatment groups were determined by Student's *t* test ($P < 0.05$). For panel C, differences between stratified exercise groups were assessed by a one-way ANOVA followed by Tukey's posttest ($P < 0.05$).

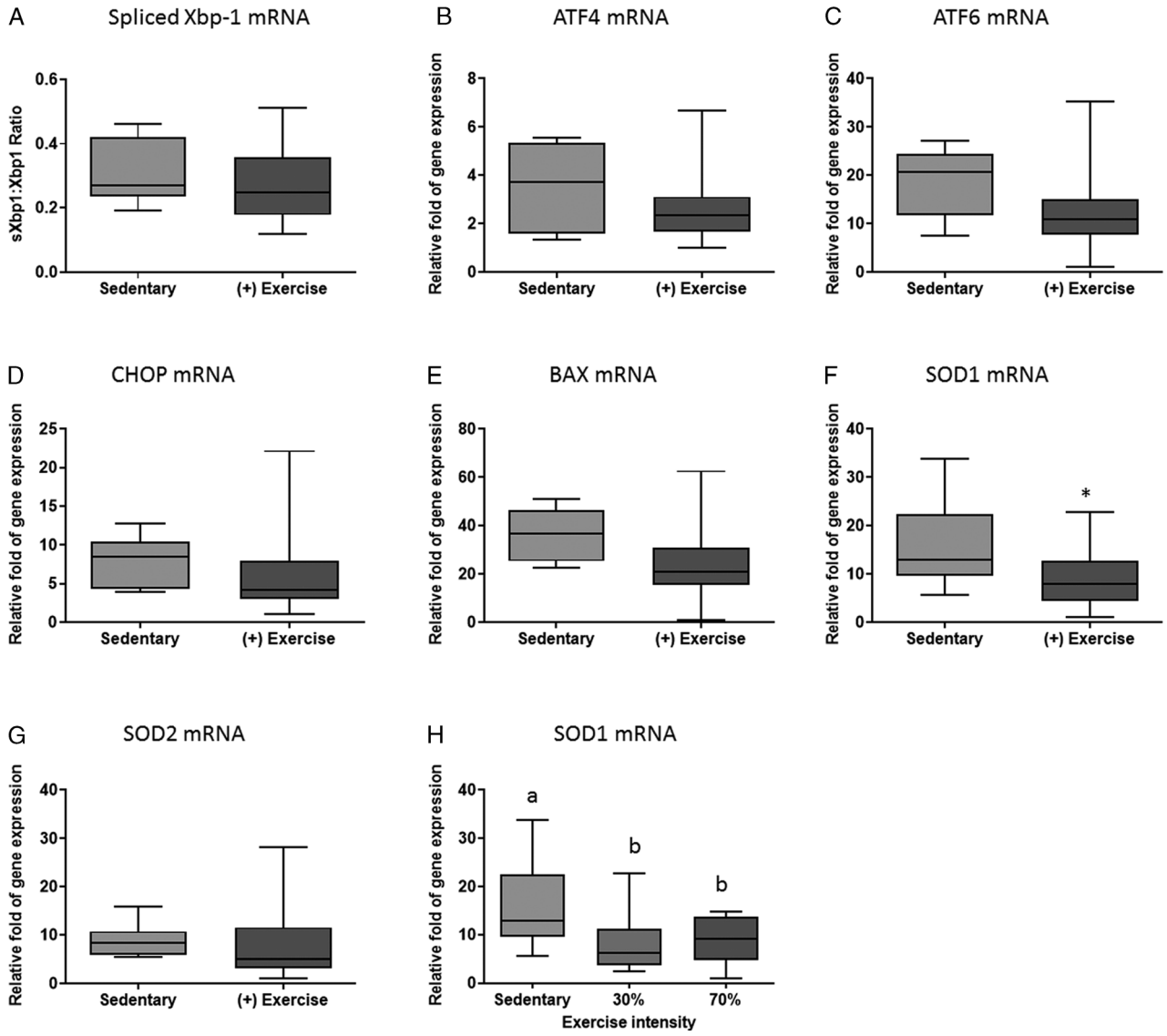

FIGURE 3—Exercise in pregnancy does not lead to ER stress or oxidative stress in the human placenta. Relative expression of Spliced Xbp-1 mRNA (A), ATF4 mRNA (B), ATF6 mRNA (C). CHOP mRNA (D), BAX mRNA (E), SOD1 mRNA (F), SOD2 mRNA (G), and SOD1 mRNA (H) between sedentary and exercising groups were assessed by real-time qPCR. Total RNA was extracted from placentae and reverse-transcribed to cDNA and normalized to GAPDH. All values were expressed as mean ± SEM. Significant differences between treatment groups were determined by Student's *t*-test ($P < 0.05$). For panel H, differences between stratified exercise groups were assessed by a one-way ANOVA followed by Tukey's posttest. Different letters represent means that are significantly different from one another ($P < 0.05$).

mild or moderate exercise in pregnancy (from 16 wk to term) did not adversely alter fetal/neonatal outcomes in a healthy pregnant population. This was evidenced by no significant changes in birth weight, fetal to placental weight ratio, and APGAR scores. This supports previous systemic reviews indicating exercise in pregnancy has no major adverse effects on maternal, fetal, or neonatal outcomes (3). Further in this cohort, the timing of delivery was not different in all groups (sedentary vs active), which removes any major confounding variables when assessing the effects of exercise on placental homeostasis. Interestingly, our studies revealed that exercise in pregnancy boosted the expression of ANG1, a marker of new blood vessel formation in the placenta. Furthermore, this increase in ANG1 expression in exercising mothers did not occur at the expense of increased hypoxia, ER, or oxidative stress in this healthy pregnant population. Collectively, this further supports that mild or moderate exercise is beneficial to maternal–fetal outcomes, whereas even mild exercise can have an added benefit of promoting placental angiogenesis. This might explain why maternal exercise is not associated with IUGR (5).

ANG1 in the placenta has been demonstrated to be a potent inducer of neovascularization in the placenta, making it a primary determinant of fetal growth (9,37,38). It is expressed in early pregnancy, localizing and secreted in both extravillous and differentiated villous cytotrophoblasts (10). It is also expressed in glandular epithelial cells, vascular cells, and macrophages allowing for crosstalk between epithelial cells and trophoblasts, critical for new blood vessel formation (9).

Interestingly, the expression of ANG1 is thought to be governed by alterations in oxygen tension (39). In human placental explants, hypoxia led to increased ANG1 expression (39), but in the current study, there was no evidence of placental hypoxia (i.e., higher VEGF or HIF1α expression). Although exercise has been linked to angiogenesis in many tissues of the body (7), the increase in placental ANG1 in pregnancy may further support the increased oxygenation needs of the maternal–fetal unit. This could be the result of the underlying increased cardiorespiratory responses in exercising mothers promoting a heightened aerobic conditioning response in the placenta. Although differences in placental ANG1 expression were not observed when stratified by mild or moderate exercise, Cohen's *d* test did reveal a large effect size for either group warranting further investigation with a randomized control trial using a larger sample size to further examine the dose-dependent effects of exercise on placental ANG1. Although an increase in ANG1 in the healthy exercising mothers would undoubtedly further benefit placental efficiency due to greater angiogenesis and blood flow, it is tempting to speculate that this could be therapeutic in pathological pregnancies such as gestational diabetes, obesity, and idiopathic IUGR whereby angiogenesis is impaired (33). A lack of change in the hypoxic markers HIF1α and VEGF in the exercising groups further supports this notion of improved placental blood flow and efficiency. Further studies are warranted to investigate the expression of ANG1 in those placental pathologies before and after exercise intervention.

Given rapid alterations in cellular metabolism (i.e., changes in bioavailability of amino acids, oxygen, and/or ATP) can lead to protein misfolding and ER stress (15), it was critical to assess if exercise-induced changes to placental angiogenesis (e.g., as indicated by higher expression of placental ANG1) were associated with augmented ER stress. This is important considering that augmented placental ER stress results in placental inefficiency leading to IUGR and gestational diabetes (17–21). Surprisingly, mild or moderate exercise in pregnancy did not lead to changes in the UPR based on measuring downstream markers of the three UPR sensors of the ER stress pathway (i.e., spliced Xbp1, ATF4, ATF). Overall, this suggests the exercise regime from midgestation (i.e., 16–20 wk) until term does not result in adverse ER homeostasis. Moreover, no change in CHOP or BAX further suggests chronic ER stress-induced apoptosis was not apparent. This is not surprising considering that exercise in pregnancy did not lead to changes in placental markers of hypoxia (i.e., HIF1α and VEGF), major drivers of placental ER stress (40,41). Any transient hypoxia occurring during physical activity is likely compensated by adaptive angiogenesis resulting from increased ANG1 expression. It should be noted that, although the steady-state expression of VEGF mRNA was unchanged, Cohen's *d* test revealed that further breakdown into the mild and moderate exercise groups showed a large effect size (1.07) relating to moderate-intensity exercise. This suggests that if a larger cohort of exercising mothers was examined, moderate exercise might boost the expression of proangiogenic VEGF, normally induced in response to placental hypoxia (42,43). Further larger randomized clinical trials are warranted to observe if placental VEGF expression increases in response to maternal exercise in a dose-dependent manner, and if moderate exercise induces VEGF in the absence or presence of placental hypoxia.

With the close approximation of the ER and mitochondria in punctate sites known as the mitochondrial-associated ER membrane, studies now indicate that integrated signaling occurs between both organelles and that ER stress can lead to both mitochondrial dysfunction and augmented oxidative stress (23). Oxidative stress is defined as an imbalance between antioxidant defenses and reactive oxygen species production (free radicals) (44). Prolonged oxidative stress can result in free radical damage, ultimately leading to mitochondrial-mediated apoptosis, which underlies pathological pregnancies such as idiopathic IUGR (19,22,24,25,44). In the current study, we examined the placental expression of the antioxidant superoxide dismutases SOD1/2 in sedentary and exercise groups to assess whether an imbalance in antioxidant expression was present due to rapid changes in angiogenesis and blood flow. We observed no change in mitochondrial-associated SOD2 but did observe a significant decrease in the expression of the SOD1, which is more ubiquitous in the cell. Even when exercise is stratified by mild- and moderate-intensity groups, both intensities of exercise lead to lower antioxidant expression. This is likely attributed to the fact that exercise in pregnancy promotes proper mitochondrial respiratory function via increases in oxidative phosphorylation and respiratory capacity. Moreover, a balance in oxidative states may remove the need or signal for antioxidant production. This would also make sense given the triggers of oxidative stress (i.e., hypoxia and ER stress) were not evident in the placentae of exercising women. Although further measures of oxidative stress need to be assessed in future studies, the findings of the current study bear clinical significance to patients, given moderate or even mild exercise in pregnancy would seem to be beneficial by reducing placental oxidative stress.

One strength of the current study is that the group demographics were not different at the outset and the exercise interventions began at the same gestational age. Limitations may be that exercise did not start during the first trimester when placentation first occurs. Commencing early exercise strategies in the first trimester or examining the placenta of women who continue exercising from before pregnancy into early pregnancy may show interesting results. Although there was a small sample size, the results may be more distinct with a larger sample size as indicated by medium to large effect sizes. We did not control for infant sex; however, males to females were equitable between groups. Finally, further indices of VEGF function (including assessment of VEGF receptor levels and/or affinity) could reveal differences between sedentary and exercising groups.

In summary, our results suggest that routine exercise during pregnancy, of mild or moderate intensities, are not associated with increased oxidative or ER stress in the placenta relative to inactivity. This further supports the notion of routine exercise as both a standard and safe practice in healthy pregnant women. Moreover, exercise in healthy pregnancy seems to boost

placental angiogenesis as revealed by dramatic increases in the expression of ANG1. This now uncovers a new benefit of mild or moderate exercise in pregnancy for the maternal–fetal unit. Future, larger-scale studies are now warranted to characterize the potential therapeutic role of ANG1 in pathological pregnancies with respect to different levels of maternal activity.

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