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Vascular effects of aerobic exercise training in rat adult offspring exposed to hypoxia-induced intrauterine growth restriction

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Key points

- Prenatal hypoxia, one of the most common consequences of complicated pregnancies, leads to intrauterine growth restriction (IUGR) and impairs later-life endothelium-dependent vascular function.
- Early interventions are needed to ultimately reduce later-life risk for cardiovascular disease.
- Aerobic exercise training has been shown to prevent cardiovascular diseases. Whether exercise can be used as an intervention to reverse the vascular phenotype of this susceptible population is unknown.
- Aerobic exercise training enhanced endothelium-derived hyperpolarization-mediated vasodilatation in gastrocnemius muscle arteries in male IUGR offspring, and did not improve nitric oxide-mediated vasodilatation in IUGR offspring.
- Understanding the mechanisms by which exercise impacts the cardiovascular system in a susceptible population and the consideration of sexual dimorphism is essential to define whether exercise could be used as a preventive strategy in this population.

Abstract Hypoxia *in utero* is a critical insult causing intrauterine growth restriction (IUGR). Adult offspring born with hypoxia-induced IUGR have impaired endothelium-dependent vascular function. We tested whether aerobic exercise improves IUGR-induced endothelial dysfunction. Pregnant Sprague–Dawley rats were exposed to control (21% oxygen) or hypoxic (11% oxygen) conditions from gestational day 15 to 21. Male and female offspring from normoxic and hypoxic (IUGR) pregnancies were randomized at 10 weeks of age to either an exercise-trained or sedentary group. Exercise-trained rats ran on a treadmill for 30 min at 20 m min^{-1}, 5 deg gradient, 5 days week^{-1}, for 6 weeks. Concentration–response curves to phenylephrine and methylcholine were performed in second order mesenteric and gastrocnemius muscle arteries, in the presence or absence of L-NAME (100 μM), MnTBAP (peroxynitrite scavenger; 10 μM), apamin (0.1 μM) and TRAM-34 (an intermediate-conductance calcium-activated potassium channel blocker; 10 μM), or indomethacin (5 μM). In adult male IUGR offspring, prenatal hypoxia had no effect on total vasodilator responses in either vascular bed. Aerobic exercise training in IUGR males, however, improved endothelium-derived hyperpolarization (EDH)-mediated vasodilatation in gastrocnemius muscle arteries. Female IUGR offspring had reduced NO-mediated vasodilatation in both vascular beds, along with decreased total vasodilator responses and increased prostaglandin-mediated vasoconstriction in gastrocnemius muscle arteries. In contrast to males, aerobic exercise training in IUGR female offspring had no effect on either vascular bed. Exercise may not prove to be a beneficial therapy for specific vascular pathways affected by prenatal hypoxia, particularly in female offspring.

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Abbreviations AUC, area under the curve; CCRC, cumulative concentration–response curve; EDH, endothelium-derived hyperpolarization; E_{max}, maximum response; GD, gestational day; IUGR, intrauterine growth restriction; MCh, methylcholine; NO, nitric oxide; PE, phenylephrine; ROS, reactive oxygen species; SK_{Ca}, small-conductance calcium-activated potassium channels.

Introduction

During a compromised pregnancy, the *in utero* environment can affect fetal growth and development resulting in intrauterine growth restriction (IUGR). Between 2 and 11% of all newborns are affected by IUGR (Olusanya, 2010), which is associated with an increased risk of neonatal morbidity (Wu *et al.* 2012) and mortality (Lawn *et al.* 2005). Importantly, IUGR has also been associated with an increased risk of chronic disease in adulthood; population-based studies have demonstrated that being born growth restricted is a risk factor for the development of ischaemic heart disease, insulin resistance, diabetes and stroke later in life (Barker *et al.* 1989; Jaquet *et al.* 2000; Class *et al.* 2014). Likewise, there is a large body of evidence that has demonstrated the association of fetal programming with adult chronic disease in a variety of animal models including dietary interventions, placental dysfunction, glucocorticoid administration, genetic models and hypoxia (reviewed in Santos & Joles, 2012; Giussani & Davidge, 2013).

Prenatal hypoxia is a critical insult causing IUGR in many pregnancy complications (Kingdom & Kaufmann, 1997). We have previously shown that IUGR following a prenatal hypoxic insult has been associated with decreased cardiac performance after ischaemia (Xu *et al.* 2006; Rueda-Clausen *et al.* 2009) and increased susceptibility to secondary stressors (e.g. ageing or a high fat diet) (Rueda-Clausen *et al.* 2009, 2011). Furthermore, IUGR has been associated with vascular dysfunction in a variety of vascular beds, affecting both male and female offspring, characterized by increased arterial wall stiffness, reduced arterial diameter, altered collagen deposition, increased vasoconstrictor capacity, decreased endothelium-derived hyperpolarization (EDH) and decreased nitric oxide (NO)-mediated vasodilatation (Payne *et al.* 2003; Mazzuca *et al.* 2010; Morton *et al.* 2010, 2011; Bourque *et al.* 2013).

Oxidative stress has been proposed as a mechanism for vascular dysfunction in IUGR (reviewed in Giussani & Davidge, 2013). Being born from a hypoxic pregnancy, independent of IUGR, has been associated with increased aortic nitrotyrosine levels (Giussani *et al.* 2012), which is a footprint of peroxynitrite, a powerful oxidant produced by the reaction of NO with superoxide (Beckman & Koppenol, 1996). Further, maternal treatment with antioxidants in hypoxic pregnancy rescued endothelial dysfunction in the offspring at adulthood (Giussani *et al.* 2012). Several other studies have shown that a vascular oxidant tone affecting NO bioavailability is functional in fetal life and that it can be modified by hypoxic conditions and by exposure to antioxidants or to agents that increase NO bioavailability (Thakor *et al.* 2010; Herrera *et al.* 2012; Kane *et al.* 2012). Thus both maternal and fetal treatments are important approaches for reducing the vascular effects of prenatal hypoxia exposure. Unfortunately, not all interventions can be administrated during pregnancy and therefore offspring who are at an increased risk of cardiovascular disease due to their prenatal environment will still require prevention or treatment options.

Aerobic exercise training has been shown to improve vascular function, and thus has been proposed as an intervention to prevent cardiovascular diseases. Exercise has been associated with an improvement of vascular function by increasing EDH-mediated vasodilatation in gastrocnemius muscle arteries from spontaneously hypertensive rats (Gündüz *et al.* 2011), increasing the expression of endothelial nitric oxide synthase in the aorta (Sessa *et al.* 1994), increasing NO-mediated vasodilatation in epicardial coronary arteries (Wang *et al.* 1993), reducing plasma endothelin-1 (Maeda *et al.* 2009) and decreasing reactive oxygen species (ROS) generation in aortic endothelial cells (Rush *et al.* 2003). Interestingly, however, in other studies it has been shown that in an animal model of vascular disease, STZ-diabetic rats, intensive exercise did not improve endothelium-dependent vasodilatation in the thoracic aorta (Zguira *et al.* 2013). Furthermore, Allen *et al.* demonstrated that exercise did not improve vascular function as assessed by brachial artery flow-mediated dilatation in subjects with type 2 diabetes and peripheral artery disease (Allen *et al.* 2014). Thus, exercise training may not be beneficial in conditions with a susceptible vascular pathology. Furthermore, the role of exercise in IUGR offspring with compromised vascular function has not yet been assessed.

Exercise training in growth-restricted animal models has been shown to improve metabolic function by suppressing glucose-stimulated insulin production and decreasing hepatic glucose production in female Sprague–Dawley rats (Garg *et al.* 2009). Moreover, Laker

et al. have shown that exercise training at weaning increased relative islet surface area and β-cell mass in growth-restricted male Wistar–Kyoto rats (Laker *et al.* 2011). These findings suggest that exercise can improve the metabolic phenotype associated with intrauterine growth restriction; however, whether exercise can be used as an intervention to reverse the vascular phenotype of this susceptible population is unknown. Thus, the aim of our study was to determine whether aerobic exercise training could be used as an early intervention strategy to improve vascular function in IUGR offspring.

Methods

Ethical approval

All procedures in this study were approved by the University of Alberta Animal Welfare Committee, and were in accordance with the guidelines of the Canadian Council on Animal Care. Our protocols followed the ARRIVE guidelines for reporting *in vivo* experiments.

Animal model

We used a model of hypoxia-induced IUGR in which we have previously demonstrated that hypoxia not only decreased offspring body weight, crown–rump length and abdominal girth, but was also associated with long-term cardiovascular effects later in life (Xu *et al.* 2006; Rueda-Clausen *et al.* 2009; Morton *et al.* 2010, 2011). Briefly, 3-month-old Sprague–Dawley ($n = 26$) rats (Charles River, Wilmington, MA, USA) were housed in the animal facility where room conditions were as follows: 35% humidity, 10 h:14 h light:dark cycle, and fed *ad libitum* with standard rodent chow. After acclimatization, rats were mated overnight. Upon confirmation of pregnancy (presence of sperm in a vaginal smear designated as gestational day (GD) 0), female rats were housed singly and then exposed to control (room air) or hypoxic (11% oxygen) conditions from GD 15 to 21. Previous findings have reported that during hypoxic conditions dams have a reduced food intake (Williams *et al.* 2005*a*; Camm *et al.* 2010). Since activity levels are also reduced in hypoxic conditions, it is likely that nutrient supply is appropriate to metabolic demand; however, it is noted that this model may consist of an interaction of nutrient restriction and hypoxia.

At the time of birth (GD 22), anthropometric parameters such as body weight, crown to rump length and abdominal girth were measured. Offspring from dams exposed to hypoxia are referred to as IUGR offspring and offspring from dams exposed to normoxia are referred to as control offspring. Litters were randomly reduced to eight pups (4 males and 4 females) to control access to maternal nutrition. Offspring were weaned at 3 weeks and the exercise intervention was started at 10 weeks of age. From each litter, two males and two females were randomly allocated to the exercise training group and two males and two females were assigned to a sedentary group. One male and one female from each group were used in a separate series of cardiac experiments and were not included in this report.

Exercise tolerance test

To determine if exercise capacity was different in control and IUGR offspring and to inform the prescription of exercise training, an exercise tolerance test was conducted. At 10 weeks of age, a subset of offspring (3 per group: control and IUGR, male and female) were familiarized with running on a motor-driven treadmill (Animal treadmill: Exer 3/6 Columbus Instruments, Columbus, OH, USA) for 10 min at 25 m min^{-1} and a 10 deg gradient for 4 days. After familiarization, each rat performed a progressive, incremental exercise test to fatigue. Testing began at a speed of 25 m min^{-1} and a 10 deg gradient for 3 min; treadmill speed was then increased to 40 m min^{-1} with the gradient held constant for 3 min. Subsequently, treadmill speed was increased progressively by 5 m min^{-1} every minute until rats were not able to continue running. Total exercise time, maximal treadmill speed and total distance run were recorded for each rat to determine maximal exercise capacity.

Exercise intervention

At 10 weeks of age, male and female offspring from hypoxic and normoxic pregnancies were randomized to an exercise training group. Offspring were progressively habituated to motor-driven treadmill running during 5 consecutive days and then exercised for 30 min at 20 m min^{-1}, 5 deg gradient, 5 days week^{-1}, for 6 weeks. This exercise protocol was adapted from Jendzjowsky & DeLorey (2011). Rats were encouraged to run with a jet of air applied to the hind-quarters. Offspring in the sedentary group were exposed to the same room environment for the same period of time as the training group.

Food intake, body weight, echo MRI

Food intake and body weight were calculated weekly from 10 to 16 weeks of age. At 15 weeks of age, whole body composition was measured in conscious offspring using EchoMRI analysis (Houston, TX, USA). The percentage of fat and lean tissue relative to body weight was calculated for each animal.

Vascular function

At 16 weeks of age, and 24 h after the last bout of exercise, offspring were anaesthetized with a single dose (1.5 ml) of inhaled isoflurane and killed by exsanguination. Vascular function was assessed using wire myography from sedentary and exercise, control and IUGR, male and female offspring. Since the vascular assessments were conducted 24 h after the last bout of exercise, we were not able to assess females at a particular day of their oestrous cycle. Second order mesenteric arteries and first or second order arteries from the medial head of the gastrocnemius muscle (defined as first or second branches of the feed artery that traverses the superficial portion of the muscle) were isolated and dissected in ice-cold physiological saline solution (in mM: 10 Hepes, 5.5 glucose, 1.56 $CaCl_2$, 4.7 KCl, 142 NaCl, 1.17 $MgSO_4$, 1.18 KH_2PO_4, pH 7.4). Mesenteric arteries were used to assess the effect of aerobic exercise training on resistance arteries important for blood pressure regulation. Gastrocnemius muscle arteries were used to assess the effect of exercise training on a vascular segment from a muscle group recruited during treadmill exercise training.

Arteries were mounted on two 40 μm wires attached to a wire myograph (DMT, Copenhagen, Denmark) to allow isometric tension recordings. Vessels were normalized and functional endothelial and smooth muscle integrity were checked as previously described (Morton *et al.* 2010). A cumulative concentration–response curve (CCRC) to phenylephrine (PE, 0.001–100 μM for mesenteric arteries and 0.00001–100 μM for gastrocnemius muscle arteries) was performed to determine the EC_{80} (concentration producing 80% of the maximum response (E_{max}) for the vasoconstrictor). Responses were normalized to artery length. For both vascular beds assessed, the maximum contractile response of smooth muscle cells was tested using a high potassium solution at the end of the protocol (in mM: 10 Hepes, 5.5 glucose, 4.9 $CaCl_2$, 124 KCl, 24 NaCl, 2.4 $MgSO_4$, 1.18 KH_2PO_4, pH 7.4).

We have previously shown that mesenteric arteries from IUGR offspring have a reduced NO component of vasodilatation while maintaining EDH, with no contribution of prostaglandins to vasodilatation (Morton *et al.* 2010). Therefore, we tested the mechanisms by which NO-induced vasodilatation was reduced in IUGR and whether this could be improved by exercise. To investigate vascular responses to the endothelium-dependent vasodilator methylcholine (MCh, 0.00001–3 μM), a CCRC was performed following preconstriction with the EC_{80} concentration of PE. CCRCs to MCh and PE were performed in mesenteric arteries with separate baths used to incubate the arteries with the following inhibitors: L-NAME (Calbiochem, Germany; 100 μM) to inhibit NO synthase activity, or Mn(III)tetrakis(4-benzoic acid)porphyrin chloride (MnTBAP, Calbiochem, Germany; 10 μM) to scavenge peroxynitrite (Batinić-Haberle *et al.* 2009).

Our primary question in any vascular bed is to address the main mechanisms of vasodilatation. Prior to our study, the mechanisms of vasodilatation had not been investigated in gastrocnemius muscle arteries of IUGR offspring. Therefore, CCRCs to MCh and PE were performed in gastrocnemius muscle arteries with separate baths used to incubate the arteries with the following inhibitors: L-NAME (100 μM), a combination of apamin (0.1 μM) and 1-[(2-chlorophenyl) diphenylmethyl]-1*H*-pyrazole (TRAM-34; 10 μM) to block small-conductance calcium-activated potassium (SK_{Ca}) channels and intermediate-conductance calcium-activated potassium (IK_{Ca}) channels, or indomethacin, a cyclooxygenase inhibitor (5 μM). Unless otherwise stated all drugs were purchased from Sigma (St. Louis, MO, USA).

Corticosterone assay

After offspring were anaesthetized and killed, a blood sample was collected by venipuncture of the inferior vena cava. Serum corticosterone levels were determined according to the manufacturer's instructions using a commercially available, colorimetric competitive enzyme immunoassay (Enzo Life Sciences, Inc., Farmingdale, NY, USA).

Statistical analyses

The data were presented as mean ± standard error of the mean (SEM). The Shapiro–Wilk test was used to assess normality of continuous data. Body weights, crown–rump length, abdominal girth, total exercise time, maximal treadmill speed and total distance run were analysed using two-sample *t* tests or a Mann–Whitney test when data were not normally distributed. This study had a two-way ANOVA design where the effect of being born growth restricted and the effect of aerobic exercise training were determined. Therefore, food intake, body weight, body composition and corticosterone levels were tested using a two-way ANOVA followed by a Bonferroni *post hoc* test. Offspring were chosen for each experimental procedure such that no two animals in one procedure were from the same litter. Seven to thirteen offspring were used in each experiment.

The effect of L-NAME, MnTBAP, apamin + TRAM-34, or indomethacin on vasoconstriction and vasodilatation was compared within each study group using a one-way ANOVA followed by a Dunnett's *post hoc* test analysis of pEC_{50} (negative log of effective concentration producing 50% of the maximum response) and E_{max} data.

The contribution of NO, ROS, EDH or prostaglandins to vasodilatation was further compared between study groups by assessment of the Δ of the vasodilatation area under the curve (AUC) with or without inhibitors. The effect of being born growth restricted and the effect of aerobic exercise training were tested using a two-way ANOVA followed by a Bonferroni *post hoc* test.

Female and male offspring data were analysed separately due to phenotypical differences. Statistical significance was defined as $P < 0.05$. All data were analysed using GraphPad Prism 5 statistical software (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Animal model

At the time of birth, male and female offspring from dams exposed to hypoxia had a lower body weight, similar crown–rump length and lower abdominal girth than offspring born from dams in normoxic conditions suggesting asymmetric growth in both sexes. However, the crown–rump length to abdominal girth ratio only reached significance in the male IUGR offspring suggesting a more subtle phenotype in the females (Table 1).

Exercise tolerance test

There were no differences regarding time and speed reached at the end of the test between control offspring and IUGR offspring in either male (time: 8.8 ± 0.4 min control *vs.* 9.6 ± 1.8 min IUGR, $P = 0.7$; speed: 56.7 ± 1.6 m min^{-1} control *vs.* 58.3 ± 9.2 m min^{-1} IUGR, $P = 0.7$) or female offspring (time: 9.2 ± 1.3 min control *vs.* 10.4 ± 1.3 min IUGR, $P = 1.0$; speed: 56.7 ± 4.4 m min^{-1} control *vs.* 68.6 ± 3.3 m min^{-1} IUGR, $P = 0.7$). Given the similar exercise capacity in all groups, the experimental exercise training intervention performed represented the same absolute work rate and relative exercise intensity in both control and IUGR groups.

Food intake, body composition and body weight

During the entire 6 weeks of the protocol, control male offspring had a lower food consumption compared to IUGR male offspring ($P < 0.0001$). Aerobic exercise, however, did not have an effect on food consumption in either male control or IUGR offspring. While aerobic exercise training decreased the percentage of fat tissue in male control offspring only ($P = 0.01$), body weight was not different between male control and IUGR, sedentary or exercised offspring (Table 2).

In female offspring, there were no differences regarding food consumption or body weight gain (Table 3). Being born growth restricted, however, was associated with an increased percentage of fat tissue ($P = 0.02$) and aerobic exercise training increased the percentage of lean tissue in both control and IUGR offspring (Table 3, $P = 0.03$).

Serum corticosterone concentration

Being born growth restricted was not associated with an increase in serum corticosterone levels in male offspring (61.6 ± 6.8 ng ml^{-1} sedentary control *vs.* 58.4 ± 7.6 ng ml^{-1} sedentary IUGR; $P = 0.67$). Aerobic exercise training tended to decrease serum corticosterone levels in both male control and IUGR offspring (45.4 ± 8.2 ng ml^{-1} exercised control and 41.9 ± 8.8 ng ml^{-1} exercised IUGR; $P = 0.0503$).

In female offspring, neither being born growth restricted nor performing aerobic exercise training had an effect on serum corticosterone levels (60.7 ± 7.9 ng ml^{-1} sedentary control; 55.7 ± 6.3 ng ml^{-1} exercised control; 58.0 ± 7.7 ng ml^{-1} sedentary IUGR; 59.7 ± 4.7 ng ml^{-1} exercised IUGR; $P = 0.8$).

Mesenteric arteries: PE-induced vasoconstriction

Compared to controls, being born growth restricted did not modify PE-induced vasoconstriction in either male ($P = 0.07$) or female offspring ($P = 0.6$). In addition, compared to sedentary animals, aerobic exercise training had no effect on maximal vasoconstriction to PE: male control offspring (E_{max}: 9.9 ± 0.4 mN mm^{-1} sedentary *vs.* 10.8 ± 0.7 mN mm^{-1} exercised), male IUGR offspring (E_{max}: 9.1 ± 0.7 mN mm^{-1} sedentary *vs.* 9.4 ± 0.7 mN mm^{-1} exercised), female control offspring (E_{max}: 7.4 ± 0.4 mN mm^{-1} sedentary *vs.* 8.1 ± 0.6 mN mm^{-1} exercised) or female IUGR offspring (E_{max}: 7.5 ± 0.3 mN mm^{-1} sedentary *vs.* 8.4 ± 0.5 mN mm^{-1} exercised).

In males, there were no changes regarding maximal vasoconstriction to PE in the presence or absence of L-NAME or MnTBAP in any of the groups (data not shown). In females, the presence of L-NAME increased maximal PE-induced vasoconstriction only in control sedentary offspring (E_{max}: 7.4 ± 0.4 mN mm^{-1} no inhibitor *vs.* 9.3 ± 0.4 mN mm^{-1} L-NAME, $P < 0.05$). Vascular sensitivity to PE, however, was unchanged following the addition of L-NAME or MnTBAP to mesenteric arteries from all groups (data not shown).

Mesenteric arteries: vasoconstriction to high potassium solution

In male and female offspring, neither IUGR nor aerobic exercise training affected maximum vasoconstriction to high potassium solution (data not shown).

Table 1. Morphological characteristics at birth of male and female offspring born from normoxic (control) dams or dams exposed to hypoxia (IUGR)

Parameter	Control male offspring	IUGR male offspring	Control female offspring	IUGR female offspring
Body weight (g)	7.2 ± 0.1	6.2 ± 0.2***	6.7 ± 0.1	5.9 ± 0.1***
Crown–rump length (mm)	45.2 ± 0.5	44.1 ± 0.5	44.1 ± 0.6	43.1 ± 0.5
Abdominal girth (mm)	47.7 ± 0.5	44.8 ± 0.7**	46.4 ± 0.6	44.2 ± 0.8*
CRL/ABG ratio	0.94 ± 0.006	0.97 ± 0.007**	0.94 ± 0.006	0.95 ± 0.01

Data presented as mean ± SEM. CRL, crown–rump length; ABG, abdominal girth. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.0001$ *vs.* control offspring.

Table 2. Food intake, body weight and body composition from male control and IUGR, sedentary and exercised offspring

Parameter	Control sedentary offspring	Control exercised offspring	IUGR sedentary offspring	IUGR exercised offspring	2-way ANOVA		
					IUGR	Exercise	Interaction
Food intake (g week^{-1})							
Week 1	34.2 ± 0.9	33.4 ± 1.6	63.2 ± 2.4	59.9 ± 5.8	<0.0001	0.35	0.44
Week 2	34.1 ± 2.9	32.1 ± 1.6	68.5 ± 4.9	59.7 ± 6.1	<0.0001	0.28	0.49
Week 3	35.7 ± 1.4	32.6 ± 0.9	66.2 ± 2.3	61.2 ± 5.7	<0.0001	0.22	0.76
Week 4	33.8 ± 0.6	34.7 ± 2.1	68.2 ± 6.2	60.8 ± 7.4	<0.0001	0.52	0.42
Week 5	34.9 ± 1.9	32.1 ± 1.4	64.2 ± 2.9	60.8 ± 5.6	<0.0001	0.40	0.93
Week 6	33.6 ± 3.1	29.9 ± 2.9	67.1 ± 3.2	71.9 ± 4.3	<0.0001	0.23	0.88
Body weight (g)							
Week 1	542.8 ± 16.9	547.6 ± 16.3	514.5 ± 13.1	546.2 ± 16.9	0.10	0.19	0.62
Week 2	562.1 ± 16.4	557.2 ± 14.0	544.7 ± 14.6	558.0 ± 19.0	0.61	0.79	0.58
Week 3	583.9 ± 18.4	569.2 ± 13.8	541.3 ± 27.4	572.8 ± 22.0	0.34	0.68	0.26
Week 4	604.0 ± 19.5	581.6 ± 16.8	578.2 ± 19.6	586.8 ± 21.0	0.59	0.72	0.43
Week 5	622.3 ± 19.8	601.5 ± 16.6	597.9 ± 22.3	596.4 ± 22.5	0.47	0.68	0.53
Week 6	640.1 ± 21.0	613.3 ± 15.9	612.4 ± 21.4	609.0 ± 26.2	0.46	0.48	0.58
Body composition (% of body weight after 6 weeks of aerobic exercise)							
Fat tissue	12.2 ± 1.2	8.4 ± 0.3*	12.5 ± 0.6	11.4 ± 1.2	0.09	0.01	0.18
Lean tissue	71.0 ± 2.0	71.1 ± 1.6	72.7 ± 2.3	72.4 ± 1.4	0.71	0.17	0.76

Data presented as mean ± SEM. *$P < 0.05$ *vs.* control sedentary after a Bonferroni *post hoc* test.

Mesenteric arteries: MCh-induced vasodilatation in male offspring

In male offspring, there was an interaction effect ($P < 0.04$) on MCh-induced vasodilatation, whereby exercise increased vasodilatation in control but decreased responses in IUGR offspring (Fig. 1*A*).

L-NAME decreased mesenteric artery sensitivity to MCh (Fig. 2*A*), demonstrating that there was an NO contribution to vasodilatation in all male groups (Table 4). Maximal vasodilatation was also decreased in the presence of L-NAME in control sedentary and IUGR exercised offspring (Fig. 2*A*; Table 4). Based on analysis of the ΔAUC for all male groups, neither being born IUGR nor exercise affected the NO contribution to vasodilatation in male offspring (Fig. 2*B*).

The addition of MnTBAP did not affect sensitivity or maximal vasodilatation of mesenteric arteries to MCh in any of the groups (Fig. 2*A*; Table 4). Moreover, analysis of ΔAUC did not show a significant group effect of either phenotype or exercise on MnTBAP treatment (Fig. 2*B*).

Mesenteric arteries: MCh-induced vasodilatation in female offspring

Neither phenotype nor aerobic exercise training had an effect on vasodilator responses to MCh in female offspring (Fig. 1*B*).

In female offspring, a significant reduction in sensitivity to MCh following the addition of L-NAME was present in control sedentary and exercised offspring as well as IUGR exercised offspring (Fig. 3*A*, Table 4). Maximal vasodilatation, however, was not reduced in the presence of L-NAME in any female group (Table 4). Analysis of the ΔAUC demonstrated that there was a significant interaction of phenotype and exercise suggesting that aerobic exercise training only improved NO-mediated vasodilatation in control offspring ($P = 0.03$; Fig. 3*B*).

Table 3. Food intake, body weight and body composition from female control and IUGR, sedentary and exercised offspring

Parameter	Control sedentary offspring	Control exercised offspring	IUGR sedentary offspring	IUGR exercised offspring	2-way ANOVA		
					IUGR	Exercise	Interaction
Food intake (g week^{-1})							
Week 1	22.2 ± 0.8	21.5 ± 0.7	21.4 ± 1.8	19.9 ± 0.9	0.22	0.38	0.68
Week 2	22.3 ± 1.1	23.2 ± 0.9	21.3 ± 0.9	20.9 ± 0.9	0.09	0.82	0.53
Week 3	21.7 ± 0.3	22.5 ± 0.6	21.9 ± 1.1	21.6 ± 1.2	0.71	0.78	0.58
Week 4	21.1 ± 0.6	21.9 ± 0.7	22.3 ± 1.5	21.5 ± 1.3	0.68	0.99	0.47
Week 5	20.9 ± 3.9	22.1 ± 3.7	21.7 ± 2.0	22.7 ± 1.4	0.78	0.66	0.96
Week 6	20.3 ± 1.5	22.8 ± 0.5	22.7 ± 0.9	23.3 ± 1.1	0.12	0.09	0.34
Body weight (g)							
Week 1	310.2 ± 5.7	310.0 ± 5.4	307.7 ± 7.1	315.4 ± 11.4	0.40	0.56	0.12
Week 2	317.5 ± 6.4	317.2 ± 6.5	309.9 ± 6.5	321.8 ± 13.9	0.87	0.51	0.50
Week 3	325.9 ± 7.2	324.9 ± 6.4	317.4 ± 6.6	327.6 ± 13.8	0.75	0.61	0.63
Week 4	332.6 ± 6.7	341.8 ± 6.4	325.3 ± 7.5	330.7 ± 13.7	0.52	0.67	0.86
Week 5	341.7 ± 8.4	343.4 ± 7.7	329.4 ± 8.9	338.0 ± 12.2	0.39	0.64	0.65
Week 6	346.3 ± 8.4	352.9 ± 6.6	339.4 ± 10.7	346.5 ± 14.6	0.85	0.84	0.64
Body composition (% of body weight after 6 weeks of aerobic exercise)							
Fat tissue	11.6 ± 1.2	9.4 ± 0.6	13.2 ± 0.9	12.3 ± 1.0	0.02	0.11	0.52
Lean tissue	73.4 ± 1.1	75.6 ± 0.6	72.2 ± 0.8	73.9 ± 0.7	0.10	0.03	0.73

Data presented as mean ± SEM.

Table 4. Mesenteric artery summary data (pEC_{50} and E_{max}) of vasodilatation to MCh from male and female, control and IUGR, sedentary and exercised offspring

Male offspring	pEC_{50}			E_{max} (%)		
	No inhibitors	L-NAME	MnTBAP	No inhibitors	L-NAME	MnTBAP
Control sedentary	7.32 ± 0.08	6.8 ± 0.14**	7.36 ± 0.08	93.16 ± 1.76	68.04 ± 8.28**	94.24 ± 2.40
Control exercised	7.56 ± 0.06	7.01 ± 0.1**	7.47 ± 0.2	96.26 ± 1.39	82.27 ± 5.96	99.03 ± 0.44
IUGR sedentary	7.56 ± 0.09	6.76 ± 0.15***	7.43 ± 0.11	93.76 ± 2.08	86.54 ± 4.25	97.73 ± 0.81
IUGR exercised	7.45 ± 0.06	6.98 ± 0.11**	7.59 ± 0.09	98.01 ± 2.56	82.87 ± 6.15*	96.81 ± 1.58
Female offspring						
Control sedentary	7.28 ± 0.09	6.76 ± 0.06*	7.49 ± 0.26	91.29 ± 3.31	86.09 ± 5.07	98.92 ± 0.83
Control exercised	7.37 ± 0.14	6.55 ± 0.12***	7.55 ± 0.09	96.47 ± 1.81	82.57 ± 6.24	99.11 ± 0.39
IUGR sedentary	7.37 ± 0.08	6.39 ± 0.51	7.49 ± 0.14	90.75 ± 3.13	71.75 ± 9.07	96.06 ± 2.51
IUGR exercised	7.57 ± 0.06	6.88 ± 0.16**	7.38 ± 0.12	93.64 ± 2.26	83.19 ± 7.21	97.26 ± 1.28

Data presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs.* artery with no inhibitor after Dunnett's *post hoc* test.

MnTBAP did not affect vascular sensitivity to MCh or maximal vasodilatation in either sedentary or exercised, control or IUGR female offspring (Fig. 3*A*; Table 4). ΔAUC analysis demonstrated that neither aerobic exercise training nor being born IUGR had an effect on the contribution of ROS to vasodilatation (Fig. 3*B*).

Gastrocnemius muscle arteries: PE-induced vasoconstriction

There were no changes regarding sensitivity to PE or maximal PE-induced vasoconstriction in the presence or absence of L-NAME, indomethacin or apamin + TRAM-34 in any of the groups (male and female, control and IUGR, sedentary and exercised; data not shown).

Gastrocnemius muscle arteries: vasoconstriction to high potassium solution

In male and female offspring, neither IUGR nor aerobic exercise training affected maximum vasoconstriction to high potassium solution (data not shown).

Gastrocnemius muscle arteries: MCh-induced vasodilatation in male offspring

In male offspring, there was an interaction of being born IUGR and aerobic exercise training ($P = 0.04$) whereby maximal vasodilatation to MCh tended to be increased following exercise in IUGR offspring (E_{max}; 76 ± 4.9% sedentary control *vs.* 61.9 ± 9.2% exercised control; 71.22 ± 8.1% sedentary IUGR *vs.* 89.9 ± 6.8% exercised IUGR).

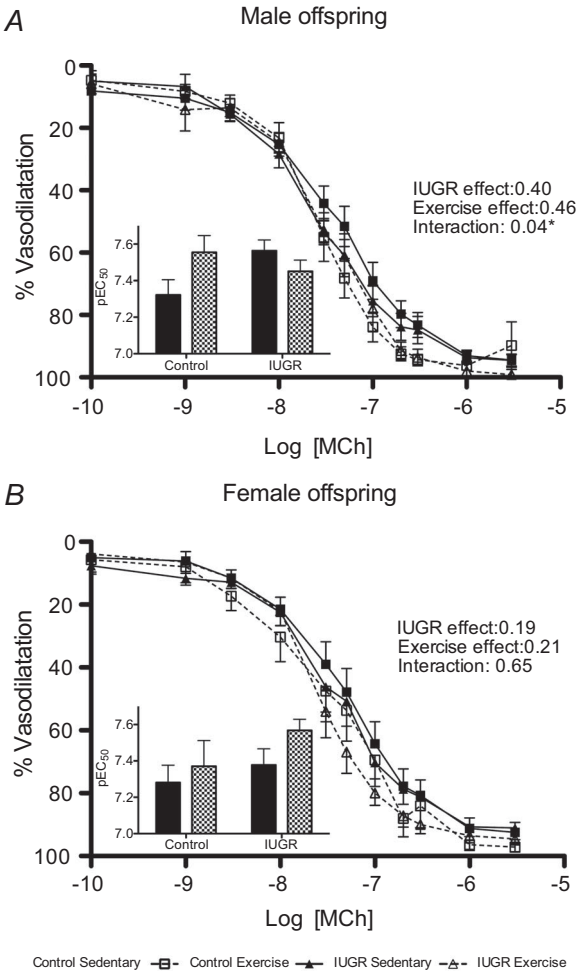

Figure 1. CCRCs to methylcholine in mesenteric arteries from male and female, control and IUGR, sedentary and exercised offspring
Percentage vasodilatation to methylcoline (MCh) in mesenteric arteries from males (*A*; $n = 8$–13) and females (*B*; $n = 8$–13). Groups include: control sedentary offspring (continuous lines, filled squares), control exercised offspring (dashed lines, open squares), IUGR sedentary offspring (continuous lines, filled triangles), and IUGR exercised offspring (dashed lines, open triangles). Data are presented as mean ± SEM and summarized as pEC_{50} in the inset bar graph figures: sedentary offspring (filled bars) and exercised offspring (hatched bars). *$P < 0.05$ for a statistically significant interaction between aerobic exercise training and IUGR in male offspring.

Following the addition of L-NAME, a decrease in the MCh E_{max} demonstrated that NO contributed to vasodilatation in gastrocnemius muscle arteries from control sedentary offspring, but not IUGR sedentary offspring. The variability in the IUGR offspring data, however, was greater and thus careful interpretation of the data is required (Fig. 4*A*, Table 5). Interestingly, NO significantly contributed to vasodilatation in both control and IUGR groups following exercise ($P < 0.01$, and $P < 0.001$; Table 5). In addition we found that EDH contributed to vasodilatation in gastrocnemius muscle arteries from control sedentary offspring ($P < 0.05$) as well as IUGR exercised offspring ($P < 0.001$, Table 5). There was no significant contribution of prostaglandins to vasodilatation in any male group. In addition, sensitivity to MCh was unaltered by any inhibitor in control or IUGR, sedentary or exercised offspring (Table 5).

Analysis of the ΔAUC showed that neither phenotype nor aerobic exercise training had an effect on either NO-mediated vasodilatation (Fig. 5*A*) or prostaglandin-mediated vascular responses (Fig. 5*C*). Further, EDH-mediated vasodilatation was increased only in IUGR exercised male offspring (Fig. 5*B*).

Gastrocnemius muscle arteries: MCh-induced vasodilatation in female offspring

Being born growth restricted impaired maximal vasodilatation to MCh in female offspring (E_{max}; 93.1 ± 0.3% control *vs.* 84.34 ± 4.15% IUGR, $P = 0.02$). Performing aerobic exercise did not improve vasodilatation in either control or IUGR female offspring.

In control sedentary females, NO contributed to vasodilatation [Fig. 4*B*, ΔpEC_{50} (no inhibitors vs. L-NAME) $P < 0.05$, ΔE_{max} (no inhibitors vs. L-NAME) $P < 0.001$; Table 5]. Following exercise, a contribution of NO to vasodilatation was still observed in control offspring [ΔE_{max} (no inhibitors vs. L-NAME) $P < 0.001$; Table 5]. While neither EDH nor prostaglandins were found to significantly contribute to vasodilatation in either sedentary or exercised control offspring, the variability of the data may have precluded the detection of more minor contributions of these vasodilator pathways. In IUGR sedentary or exercised female offspring, there were no significant changes in sensitivity to MCh or maximal vasodilatation after the addition of any inhibitor (Fig. 4*B*; Table 5). As observed in arteries from male IUGR offspring, however, the variability of the data was greater than in control groups complicating the interpretation.

Analysis of the ΔAUC demonstrated that being born growth restricted was associated with a reduced NO vasodilator component ($P = 0.007$, Fig. 6*A*), an increased involvement of prostaglandin-mediated vasoconstriction ($P = 0.002$, Fig. 6*C*) and had no effect on EDH-mediated vasodilatation (Fig. 6*B*).

Table 5. Gastrocnemius muscle artery summary data (pEC_{50} and E_{max}) of vasodilatation to MCh from male and female, control and IUGR, sedentary and exercised offspring

	pEC_{50}				E_{max} (%)			
	No inhibitors	L-NAME	Apamin + Tram-34	Indomethacin	No inhibitors	L-NAME	Apamin + Tram-34	Indomethacin
Male offspring								
Control sedentary	7.51 ± 0.16	7.73 ± 0.63	7.52 ± 0.09	7.37 ± 0.18	76.03 ± 4.89	29.09 ± 6.48***	55.96 ± 3.19*	80.25 ± 7.66
Control exercised	7.73 ± 0.26	7.73 ± 0.6	7.44 ± 0.15	7.05 ± 0.23	58.88 ± 10.68	17.72 ± 6.25**	39.16 ± 6.72	67.17 ± 9.63
IUGR sedentary	7.60 ± 0.14	8.04 ± 0.61	7.63 ± 0.14	7.21 ± 0.08	71.22 ± 8.12	36.6 ± 21.65	67.65 ± 0.81	87.17 ± 5.28
IUGR exercised	7.37 ± 0.09	ND	7.19 ± 0.17	7.57 ± 0.13	89.92 ± 6.82	46.06 ± 4.87***	42.03 ± 7.13***	86.63 ± 4.17
Female offspring								
Control sedentary	7.61 ± 0.07	7.11 ± 0.18*	7.45 ± 0.08	7.23 ± 0.08	93.4 ± 3.47	32.61 ± 12.25***	70.13 ± 6.33	83.84 ± 5.67
Control exercised	7.53 ± 0.12	7.59 ± 0.42	7.29 ± 0.13	6.87 ± 0.34	92.8 ± 5.49	36.85 ± 16.61**	72.10 ± 3.97	70.04 ± 7.17
IUGR sedentary	7.29 ± 0.11	7.22 ± 0.19	7.1 ± 0.1	7.43 ± 0.08	84.34 ± 4.15	51.39 ± 17.62	55.16 ± 9.54	92.81 ± 4.24
IUGR exercised	7.56 ± 0.13	7.21 ± 0.30	7.28 ± 0.11	8.1 ± 0.22	70.79 ± 10.87	39.26 ± 15.26	69.3 ± 6.24	92.11 ± 2.48

Data presented as mean ± SEM. ND, not determined. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* artery with no inhibitor after Dunnett's *post hoc* test.

Discussion

Many strategies have been proposed to prevent cardiovascular diseases; however, despite large volumes of research that have attempted to discover cardiovascular protective mechanisms, exercise is one of the most practical and effective preventive treatments that has been found to date (Powers *et al.* 2008). To our knowledge, this is the first study to investigate the vascular effects of aerobic exercise in offspring born growth restricted. Using a hypoxia-induced IUGR model, we found that female IUGR offspring had reduced NO-mediated vasodilatation in both mesenteric and gastrocnemius muscle arteries while in male IUGR offspring NO-mediated vasodilatation was reduced only in the gastrocnemius muscle arteries. Furthermore, we demonstrated that female IUGR offspring had a decreased vasodilator response in gastrocnemius muscle arteries and there was an increase in prostaglandin-mediated vasoconstriction. Interestingly, while in control offspring aerobic exercise training increased vasodilator responses in mesenteric arteries from males and enhanced NO-modulation in mesenteric arteries from females; in IUGR offspring, aerobic exercise training only improved EDH-mediated vasodilatation in gastrocnemius muscle arteries from males.

A hypoxic insult during the last third of pregnancy has been associated with vascular dysfunction in the offspring. Prenatal hypoxia in rats has been associated with increased fetal aortic thickness (Camm *et al.* 2010; Giussani *et al.* 2012), and with increased vascular peroxynitrite generation (Giussani *et al.* 2012), which can lead to endothelial dysfunction. In addition, it has been shown in postnatal day 1 rats that there was an enhanced femoral vasoconstrictor response to phenylephrine while this response in the carotid arteries was decreased, demonstrating that neonatal vascular adaptations following a hypoxic insult are vascular bed specific and may be pivotal in increasing blood flow to vital organs such as the brain while reducing blood flow to other organs (Williams *et al.* 2005*a*). In both male and female IUGR offspring as adults, the contribution of NO to mesenteric artery vasodilatation was reduced (Williams *et al.* 2005*b*; Morton *et al.* 2010, 2011; Giussani *et al.* 2012). Only female IUGR offspring demonstrated an enhanced myoendothelial gap junction/EDH-mediated vasodilatation in mesenteric arteries as a possible compensatory mechanism to maintain vascular function (Morton *et al.* 2010). In the current study, EDH-mediated vasodilatation was not significantly enhanced in gastrocnemius muscle arteries from either male or female IUGR offspring.

The present study also demonstrated that exposure to hypoxia *in utero* was associated with an enhanced prostaglandin-mediated vasoconstriction in gastrocnemius muscle arteries in IUGR female offspring. The

mechanisms that have been associated with increased prostaglandin-mediated vasoconstriction include: upregulation of prostaglandin H synthase 1 and 2 (PGHS 1 and 2); activation of PGHS by ROS and enhanced activation of thromboxane receptors. Interestingly, increased prostaglandin-mediated vasoconstriction has been associated with conditions of vascular pathologies such as hypertension (Vanhoutte *et al.* 2005) and diabetes (Matsumoto *et al.* 2007) as well as ageing (Stewart *et al.* 2000). The increased PGHS-dependent constriction

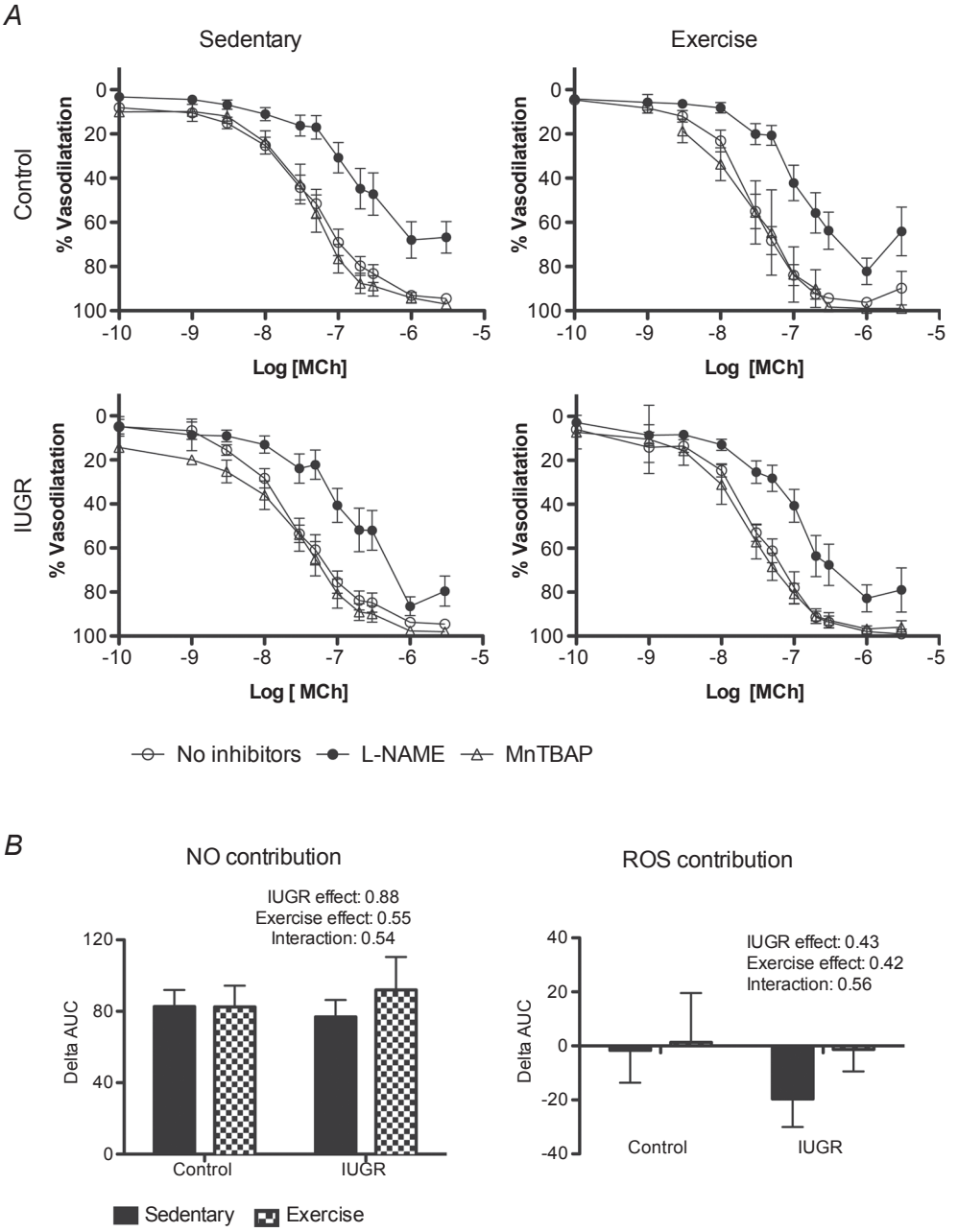

Figure 2. CCRCs to methylcholine in mesenteric arteries from male control and IUGR, sedentary and exercised offspring in the presence or absence of l-NAME or MnTBAP
A, percentage vasodilatation to MCh in mesenteric arteries from males ($n = 7$–13). Responses to methylcholine in control and IUGR animals in sedentary or exercised groups are shown in the absence of inhibitors (open circles), in the presence of L-NAME (filled circles), or in the presence of MnTBAP (open triangles). *B*, summary data of the ΔAUC in vessels incubated with no inhibitor or L-NAME, equivalent to the contribution of NO to the vasodilator response to MCh, and in vessels incubated with no inhibitor or MnTBAP, equivalent to the contribution of ROS to the vasodilator response to MCh. Data are presented as mean ± SEM.

observed in our study of young female adults exposed to prenatal hypoxia suggests the development of vascular pathology prior to overt disease.

Our data suggest that the mechanisms that lead to endothelial dysfunction in IUGR offspring differ according to sex, with a greater negative impact on female IUGR offspring compared to male IUGR offspring. Contrary to our findings, Ozaki *et al.* found that both hypertension and vascular dysfunction in the offspring of protein-restricted dams was predominant among male offspring (Ozaki *et al.*

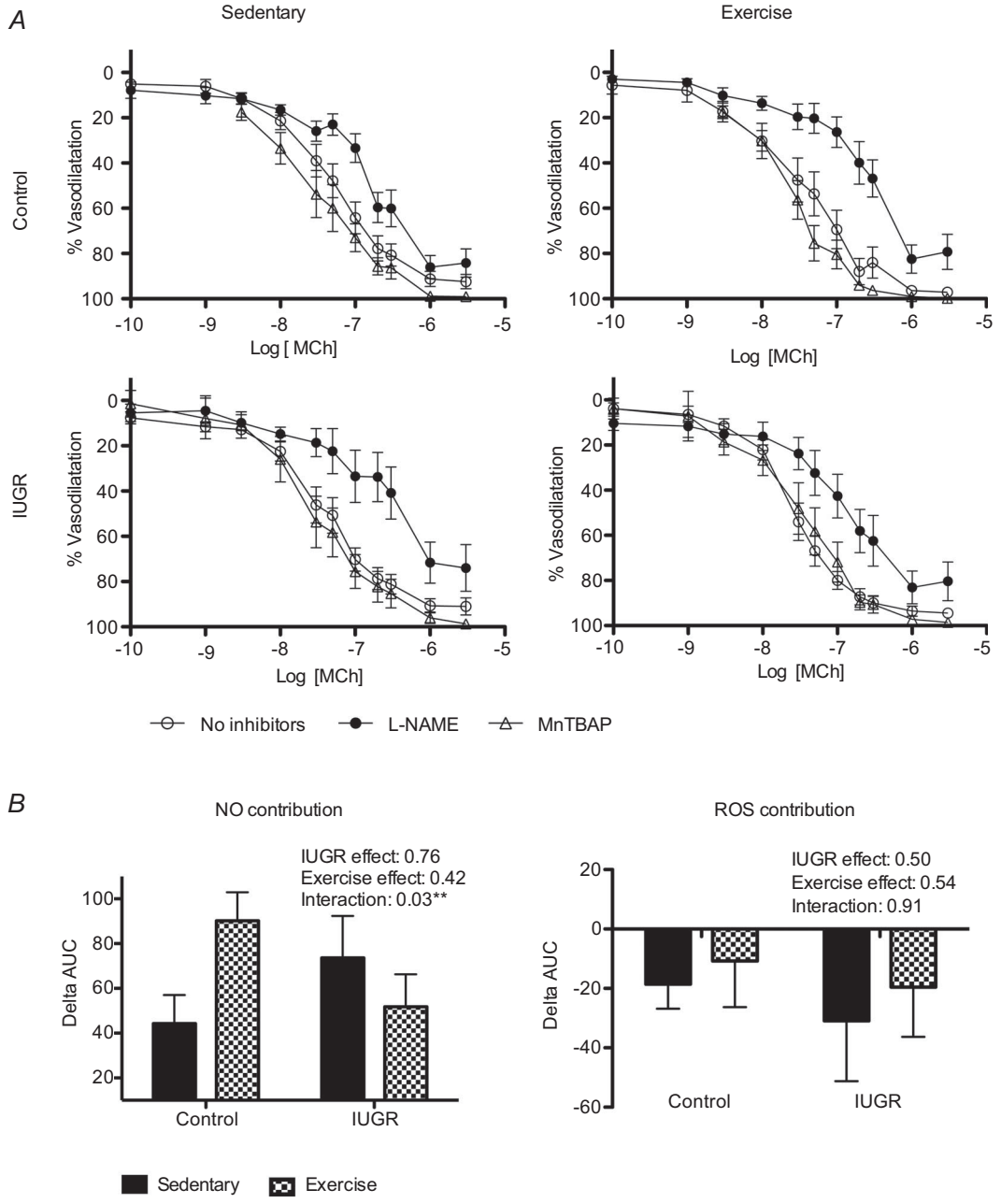

Figure 3. CCRCs to methylcholine in mesenteric arteries from female control and IUGR, sedentary and exercised offspring in the presence or absence of l-NAME or MnTBAP
A, percentage vasodilatation to MCh in mesenteric arteries from females ($n = 7$–13). Responses to methylcholine in control and IUGR animals in sedentary or exercised groups are shown in the absence of inhibitors (open circles), in the presence of L-NAME (filled circles), or in the presence of MnTBAP (open triangles). *B*, summary data of the ΔAUC in vessels incubated with no inhibitor or L-NAME, equivalent to the contribution of NO to the vasodilator response to MCh, and in vessels incubated with no inhibitor or MnTBAP, equivalent to the contribution of ROS to the vasodilator response to MCh. Data are presented as mean ± SEM. $^{**}P < 0.01$ for a statistically significant interaction between aerobic exercise training and IUGR in the presence of L-NAME.

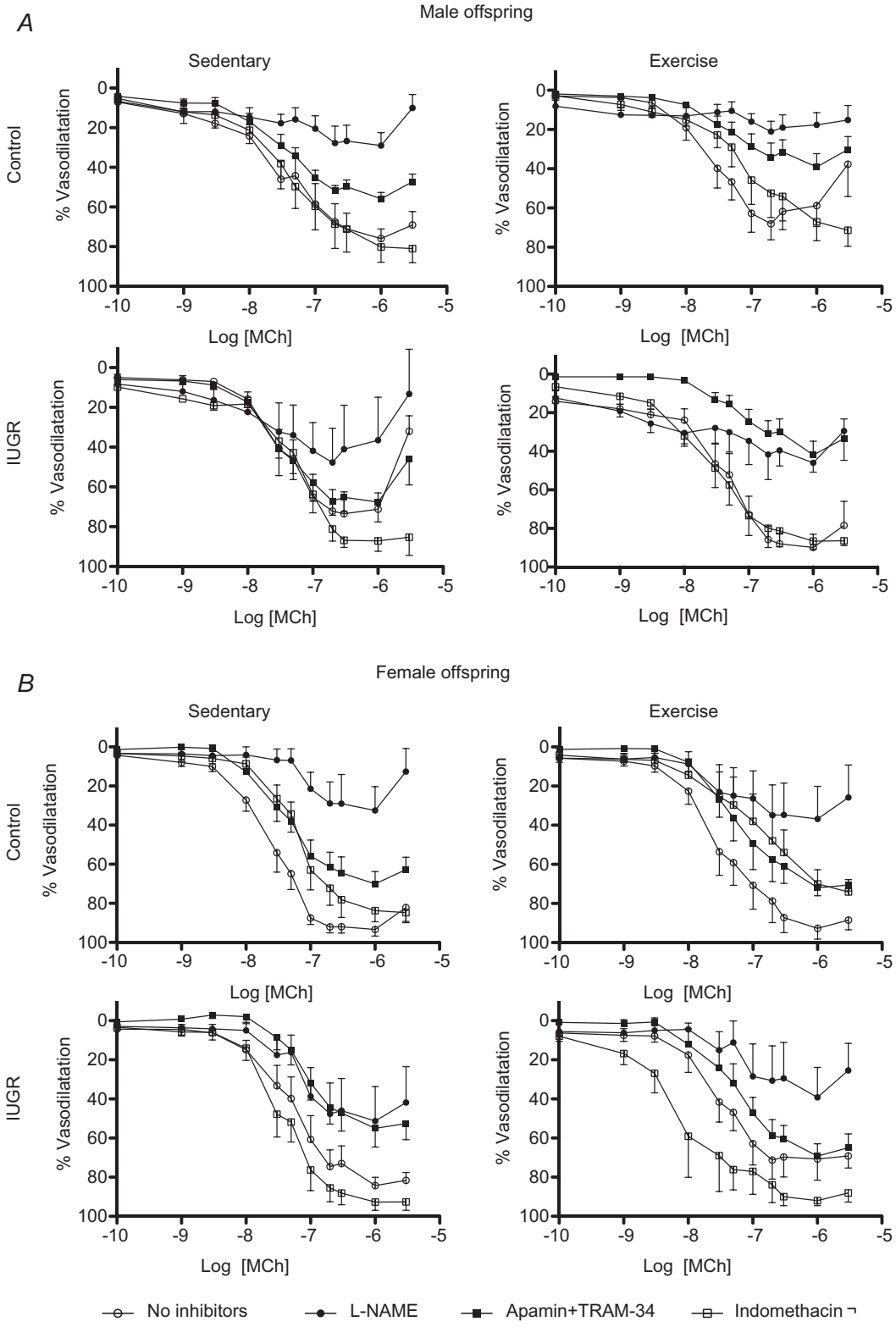

Figure 4. CCRCs to methylcholine of gastrocnemius muscle arteries from male and female, control, IUGR, sedentary and exercised offspring in the presence or absence of l-NAME, apamin + TRAM-34, or indomethacin

Percentage vasodilatation to MCh in gastrocnemius muscle arteries from males (*A*, $n = 4$–8) and females (*B*, $n = 4$–7). Responses to methylcholine in control and IUGR animals in sedentary or exercised groups are shown in the absence of inhibitors (open circles), in the presence of L-NAME (filled circles), in the presence of apamin + TRAM-34 (filled squares), or in the presence of indomethacin (open squares). Data are presented as mean ± SEM.

2001). In a model of uteroplacental insufficiency fetal programming, however, female offspring exhibited increased arterial wall stiffness in uterine and renal arteries in the absence of altered vascular reactivity in either mesenteric or femoral vascular beds (Mazzuca *et al.* 2012). Further, previous findings from our laboratory have determined that in aged female IUGR offspring NO-mediated, flow-induced vasodilatation was reduced and there was a predominant EDH-mediated vasodilator component compared to male IUGR offspring (Morton *et al.* 2011). In addition, with ageing the mechanisms involved in preserving EDH-mediated vasodilatation were

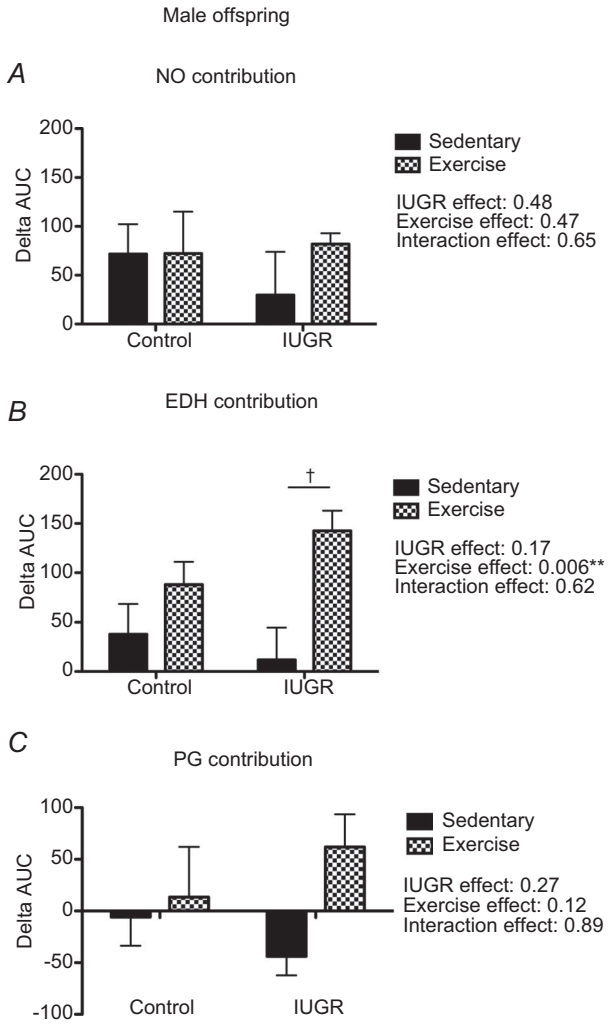

Figure 5. Nitric oxide, EDH and prostaglandin contribution to gastrocnemius muscle artery vasodilatation in male control and IUGR, sedentary and exercised offspring
Summary data of vasodilator responses to MCh in gastrocnemius muscle arteries ($n = 4$–8). *A*, summary data of the ΔAUC in vessels incubated with no inhibitor or with L-NAME, equivalent to the contribution of NO to vasodilator responses to MCh. *B*, summary data of the ΔAUC in vessels incubated with no inhibitor or with apamin + TRAM-34, equivalent to the contribution of EDH to vasodilatation. *C*, summary data of the ΔAUC in vessels incubated with no inhibitor or with indomethacin, equivalent to the contribution of prostaglandins (PG) to vasodilatation. Data are presented as mean ± SEM; sedentary offspring (filled bars), exercised offspring (hatched bars). $^{**}P < 0.01$ for a statistically significant exercise effect. $^{\dagger}P < 0.05$ *vs.* sedentary IUGR offspring after a Bonferroni *post hoc* test.

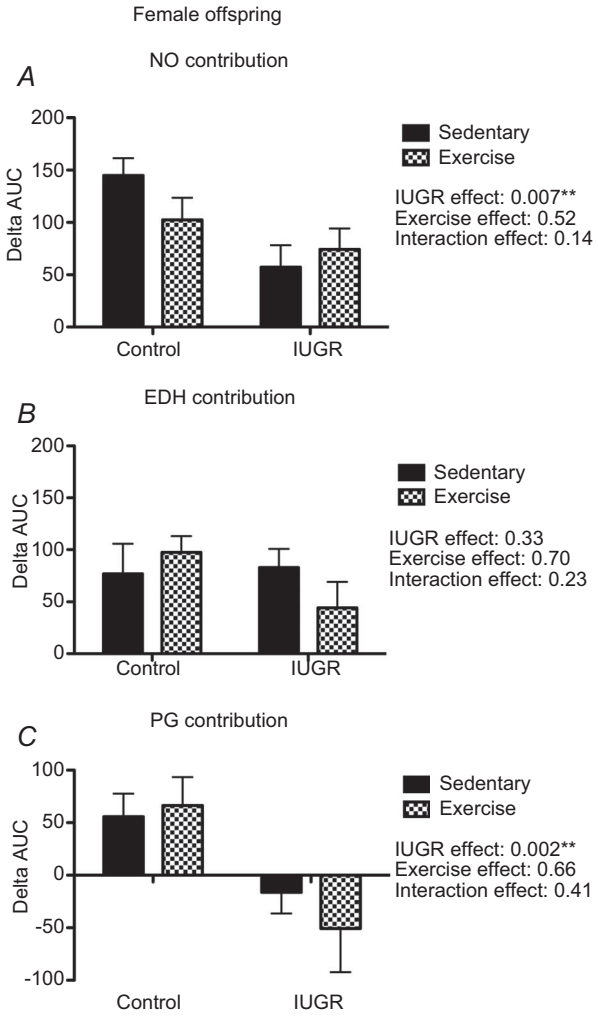

Figure 6. Nitric oxide, EDH and prostaglandin contribution to gastrocnemius muscle artery vasodilatation in female, control and IUGR, sedentary and exercised offspring
Summary data of vasodilator responses to MCh in gastrocnemius muscle arteries ($n = 4$–7). *A*, summary data of the ΔAUC in vessels incubated with no inhibitor or with L-NAME, equivalent to the contribution of NO to the vasodilator responses to MCh. *B*, summary data of the ΔAUC in vessels incubated with no inhibitor or with apamin + TRAM-34, equivalent to the contribution of EDH to vasodilatation. *C*, summary data of the ΔAUC in vessels incubated with no inhibitor or with indomethacin, equivalent to the contribution of prostaglandins (PG) to vasodilatation. Data are presented as mean ± SEM; sedentary offspring (filled bars), exercised offspring (hatched bars). Being born growth restricted was associated with a reduced NO vasodilator component, an increased involvement of prostaglandin-mediated vasoconstriction, and had no effect on EDH-mediated vasodilatation. $^{**}P < 0.01$ for a statistically significant IUGR effect.

different between sexes; a decrease in the contribution of myoendothelial gap junctions to vasodilatation has been found in aged male IUGR offspring compared to aged female IUGR offspring (Morton *et al.* 2010). It has also been reported that a hypoxic insult during pregnancy increased myogenic tone in mesenteric arteries in only male IUGR offspring (Hemmings *et al.* 2005). We can conclude that the type and severity of the insult that create a phenotype, the vascular bed assessed, as well as the sex of the offspring all play a role in the presentation of vascular dysfunction later in life following a compromised pregnancy.

Aerobic exercise training has been shown to improve vascular function by increasing NO bioavailability (reviewed in Green *et al.* 2004) and by reducing ROS (reviewed in Campos *et al.* 2013). Our findings in control female offspring showed that aerobic exercise training enhanced NO-mediated vasodilatation without affecting the total vasodilatation response in mesenteric arteries. This could be due to a reduction in other vasodilatory pathways such as EDH or prostaglandins; however, there is also the potential for redundancy of vasodilator mechanisms.

Our results regarding ROS did not demonstrate improved vasodilatation with a peroxynitrite scavenger. The impairment of NO-mediated vasodilatation observed in female IUGR offspring, therefore, was not likely to be secondary to a reduction in NO bioavailability via scavenging by superoxide and might instead be due to a decrease in NO production. Further experiments are needed in order to assess this observation.

In accordance with our findings, it has previously been demonstrated that exercise training was associated with an increase in EDH-mediated vasodilatation in animal models of disease such as hypertension (Yen *et al.* 1995; Gündüz *et al.* 2011) and diabetes (Minami *et al.* 2002). The mechanism by which this improvement may occur is not completely understood and remains under investigation. Exercise training, however, has been shown to increase whole cell potassium current activation and improve functional gating of calcium-activated potassium channels without affecting protein expression in coronary arteries from diabetic dyslipidaemic pigs (Mokelke *et al.* 2003). In addition, Milkau *et al.* determined that EDH-mediated vasodilatation was crucial for active hyperaemia, and was mediated through the activation of endothelial SK_{Ca} channels and spread along the vascular wall by connexin 40 endo–endo and myo–endo gap junctions (Milkau *et al.* 2010). Upregulation of SK_{Ca} and connexin 40 or improvement of their function could, therefore, be involved in the beneficial effect of aerobic exercise training in the vasculature observed in male IUGR offspring.

Aerobic exercise as an intervention in rodents has been suggested to chronically activate stress responses (Arida *et al.* 2004; Leasure & Jones, 2008). Moreover, it has been shown that being born growth restricted was associated with increased mineralocorticoid mRNA expression in the hippocampus and increased free corticosterone in plasma after a 30 min restraint stress test (Lesage *et al.* 2002). In the present study we found that neither exposure to hypoxia *in utero* nor aerobic exercise training was associated with changes in corticosterone levels. These findings suggest that aerobic exercise training as an intervention in a hypoxia-induced IUGR animal model is not a secondary stressor.

A variety of training protocols (voluntary *vs.* forced exercise; frequency; intensity and time) have been utilized to investigate physiological adaptations to exercise. Training-mediated changes in vascular function appear to be sensitive to the intensity and volume of exercise training (Green *et al.* 2011; Jendzjowsky & DeLorey, 2013). Our protocol was based on findings from Jendzjowsky & DeLorey (2011) where the authors calculated that the given exercise protocol corresponded to approximately 50% of maximal exercise tolerance in rodents. Moreover, the chosen training protocol is in alignment with the American College of Sports Medicine guidelines for exercise testing and prescription (ACSM, 2013) which recommend 150 min $week^{-1}$ of moderate intensity aerobic activity to delay premature mortality and reduce the risk of chronic diseases (including cardiovascular diseases). In the present study, exercise tolerance was not different between control and IUGR offspring and therefore exercise training was completed at the same absolute work rate and relative intensity in all groups. Thus, group and sex differences in the vascular responses to exercise training are not attributable to differences in the training stimulus.

We proposed aerobic exercise as an intervention to prevent the development of endothelial dysfunction later in life in young offspring born from dams exposed to hypoxia. Aerobic exercise as an intervention, however, has also been studied earlier in development (i.e. during pregnancy) to improve both maternal and offspring outcomes. Specifically, Vega *et al.* have shown in an animal model of maternal obesity that aerobic exercise improved metabolic function in the dams while it decreased leptin and triglyceride serum levels, and decreased fat deposition in male offspring (Vega *et al.* 2013). Maternal aerobic exercise training had no effect on female offspring. It has been proposed that the plasticity of any physiological system declines with age. Furthermore, ageing accumulates inadequate responses to new environmental challenges (Hanson *et al.* 2011). Thus, intervention strategies should focus not only on the timing, but also on the insult creating a phenotype and which population may benefit from the approach.

Our findings have demonstrated that hypoxia during the last third of pregnancy affects offspring birth weight and abdominal girth in both male and female offspring. The IUGR offspring, however, underwent a rapid growth

phase and their body weights were not different compared to control offspring by the beginning of the aerobic exercise intervention at ten weeks of age.

In accordance with previous studies (Desai *et al.* 2007; Fukami *et al.* 2012), our findings show that male IUGR offspring had an increased appetite. Moreover, we found a disparity between increased appetite and weight gain in male control and IUGR offspring. Interestingly, fat tissue accumulation was not different among the groups, suggesting that in male IUGR offspring there could be metabolic alterations associated with this phenomenon. It has already been established that IUGR is associated with a reduction in proteins associated with nutrient absorption and transport as well as energy metabolism in the fetal gut, predisposing the gut to metabolic defects during gestation and neonatal periods (Wang *et al.* 2014). These alterations might make them prone to obesity later in life. Although in our study IUGR offspring weight was not increased compared to control offspring, we have previously described that IUGR offspring have an increased susceptibility to develop intra-abdominal fat deposition, insulin resistance, impaired glucose tolerance and dyslipidaemia with a high fat diet compared to control offspring (Rueda-Clausen *et al.* 2011). Since female IUGR offspring had a higher percentage of fat tissue compared to female control offspring we suggest that the catch-up growth observed in female offspring was due to fat tissue accumulation, suggesting a greater metabolic efficiency.

In conclusion, exposure to hypoxia *in utero* was associated with decreased NO-mediated vasodilatation in female offspring in both mesenteric and gastrocnemius muscle arteries. An increase in prostaglandin-mediated vasoconstriction in gastrocnemius muscle arteries was also observed in female IUGR offspring. Our data suggest that exercise enhanced NO-mediated vasodilatation in female control offspring but not IUGR offspring. Furthermore, exercise improved EDH-mediated vasodilatation only in male IUGR offspring. Results from the present study highlight that understanding the mechanisms by which exercise impacts specific vascular beds in a susceptible population is essential. Exercise may not prove to be a beneficial instrument for specific vascular pathways affected by prenatal hypoxia, particularly in female offspring.

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Additional information

Competing interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Author contributions

L.M.R. contributed to the conception and design of the experiments, collection of the data, analysis and interpretation of the data, and drafting the article. J.S.M. contributed to the conception and design of the experiments, collection of the data, analysis and interpretation of the data, and revising the manuscript critically. R.K. contributed to the collection of the data, interpretation of the data, and revising the manuscript critically. D.S.D. contributed to the conception and design of the experiments, interpretation of the data, and revising the manuscript critically. S.T.D. contributed to the conception and design of the experiments, analysis and interpretation of the data, and revising the manuscript critically. The experiments were carried out in the laboratory of S.T.D at the University of Alberta. All authors approved the final version of the manuscript. All the people listed as authors qualify for authorship.

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