The Effects of Maternal Exercise During Pregnancy on the Vascular Smooth Muscle Cell Function of Offspring

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THE EFFECTS OF MATERNAL EXERCISE 
DURING PREGNANCY ON THE VASCULAR 
SMOOTH MUSCLE CELL FUNCTION OF OFFSPRING 

By 
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A Thesis Submitted in Partial Fulfillment 
Of the Requirements for a Degree with Honors 
(Dietetics and Nutrition, Fitness and Health) 

The College of Health and Human Sciences 
Purdue University 
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Clifford 1
ABSTRACT

Cardiovascular disease is the leading cause of death worldwide. It has been well documented that exercise decreases the occurrence of cardiovascular events. Since the risk of cardiovascular disease development begins in the intrauterine environment, it is critical to assess the role of exercise in the prevention of cardiovascular disease in the developing offspring. Previous studies have linked increased disease susceptibility with decreased vascular function. Therefore, the purpose of this investigation was to test the hypothesis that maternal exercise during gestation does not have an effect on the vascular smooth muscle cell function of the offspring. Twenty primiparous crossbred gilts were artificially inseminated and randomized into sedentary and exercise groups. The exercised group (n=4) performed 15 weeks of treadmill running. Contrary to our hypothesis, the results demonstrated that offspring from exercised dams had reduced vascular smooth muscle relaxation induced by sodium nitroprusside. This study was the first to investigate the impact of maternal exercise on offspring atherosclerotic disease susceptibility throughout life and potentially determine the origins of cardiovascular disease. Future studies should investigate the underlying causes of these alterations in the vascular smooth muscle cells.
PURPOSE

The purpose of this investigation was to determine the effects of maternal exercise during pregnancy on the vascular smooth muscle cell function of offspring.

LITERATURE REVIEW

Cardiovascular Disease and Atherosclerosis

Cardiovascular disease is the leading cause of death in the United States, Europe, and Asia.\(^1,2\) Alone, it is responsible for the death of over 19 million individuals.\(^1,2\) Atherosclerotic cardiovascular disease is an inflammatory disease caused by the build up of low-density lipoproteins within the artery walls.\(^1,2\) Increased inflammation causes development of atherosclerosis, but also leads to unstable plaque formation within the artery walls.\(^3\) Most commonly, a rupture of plaque causes death by obstructing blood flow.\(^3\) Although symptoms of atherosclerosis may take many years to evolve, fatty streaks and lesions have been noted in young children and even infants.\(^1,4\) The risk factors for atherosclerosis have been well established and include hypercholesterolemia, hypertension, smoking, male gender, obesity, age and diabetes mellitus.\(^3,5,6\) Research has clearly demonstrated that a healthy diet also plays a pivotal role in reducing atherosclerotic disease risk.\(^3\) In addition, increased physical activity and exercise have a high association with decreased susceptibility.\(^7\) Even though genetics and lifestyle factors play a large role in disease susceptibility, there are several underlying causes of disease that have yet to be elucidated.
The Vascular Endothelium and Smooth Muscle

Several studies demonstrate the critical link between vascular health as an underlying mediator in atherosclerotic disease susceptibility. It is well known that alterations in vascular function serve as an index for atherosclerotic disease. Alterations in arterial relaxation and constriction play a role in several diseases, especially atherosclerotic disease. A basic understanding of the anatomy of the blood vessels provides evidence to the overall hypothesis of the effects of maternal exercise on vascular function. The arteries throughout the body are made up of three layers. Each layer plays a separate role in the maintenance of blood flow throughout the arteries. The following sections will focus on the two inner layers, the tunica intima and the tunica media, due to their large role in the disease process.

Endothelial Cells

The tunica intima is made up a single layer of endothelial cells, which play a major role in regulating vascular tone. It is generally accepted that the vascular endothelium has a key role in the maintenance of vascular health because it controls the inner walls of the arteries. This single layer of endothelial cells synthesizes and releases vasoregulatory substances that act upon the underlying vascular smooth muscle. Prostacyclin (PGI2), nitric oxide (NO) and endothelium derived hyperpolarizing factor (EDHF) are three substances that are produced within the endothelial cell and cause vascular smooth muscle relaxation. On the other hand, angiotensin II and endothelin-1 are synthesized by the endothelial cell layer and cause vascular smooth muscle constriction.
Of the many substances synthesized in the vascular endothelium, nitric oxide is thought to play the most significant role in the regulation of vascular smooth muscle tone and the atherosclerotic process.\(^7,8\) Therefore, the following discussion will primarily focus on the signaling cascade involved in the synthesis of nitric oxide. NO, which is synthesized by endothelial cells, is made from the oxidation of L-arginine to L-citrulline which catalyzes Nitric Oxide Synthase (NOS) (demonstrated in figure 1).\(^8\) Several factors determine the activity of endothelial NOS (eNOS).\(^8\) Interestingly, exercise stimulates eNOS through increased blood flow resulting in increased shear stress on the arterial wall. Exercise induced shear stress initiates eNOS activity, which results in increased NO release to the vascular smooth muscle.\(^8\) Regular physical activity inhibits atherogenic occurrences and has proven to maintain endothelial function.\(^7\) In addition, it is well established that a lower availability of NO directly leads to decreased vascular function and increased disease susceptibility.\(^5,6,7\) Hypertension, insulin resistance, atherosclerosis and diabetes mellitus have all been noted in patients with endothelial dysfunction.\(^5\)

*Smooth Muscle Cells*

The middle layer of the arteries is made up of smooth muscle cells.\(^8\) The smooth muscle cells control vascular tone throughout the body.\(^8\) In the body, chemicals released by the endothelial layer act upon the vascular smooth muscle, causing relaxation or constriction of smooth muscle cells. Vessel relaxation occurs following a cascade of events where NO enters into the smooth muscle cells. Once NO enters the cell, guanylyl cyclase (GC) is initiated.\(^8\) The activation of the GC enzyme catalyzes the conversion of GTP to cyclic GMP.\(^8\) This, in turn, lowers intracellular calcium and leads to vascular
smooth muscle relaxation. Relaxation of the vascular smooth muscle results in an increase in blood vessel diameter and blood flow.\(^6\) A decrease in blood vessel diameter exists in hypertensive patients and can lead to cardiovascular events. It is clear that the function of the vascular endothelium and smooth muscle play a significant role in atherosclerotic disease susceptibility in human populations. Therefore, recent research has focused on the blood vessels for a further understanding of cardiovascular disease.

*In Research*

Several studies have been completed to further elucidate the underlying mechanisms of atherosclerotic disease. In research, a measurement of vascular smooth muscle cell function is often made using exogenous NO. Exogenous NO acts upon the smooth muscle independent of the endothelial cells. The use of animal models offers a detailed examination of vascular health.\(^5\) One common method in assessing vascular health is in-vitro wire myography, which is completed by the use of harvested blood vessels from animals.\(^6\) In vitro-wire myography utilizes vasoactive substances, such as Bradykinin or Acetylcholine, in order to measure the blood vessel’s range of dilation.\(^6\) Both of these vasodilators act by initiating a cascade of events that relax the blood vessel. Bradykinin increases NO release from the endothelial cells and stimulates eNOS enzymes. In vitro wire myography also determines the function of the endothelial cells by activating the production of NO through the eNOS enzyme.\(^5,8\) In addition, sodium nitroprusside (SNP) is often used during in-vitro wire myography experiments to measure vascular smooth muscle function.\(^6\) By understanding the function of harvested arteries, additional mechanisms of disease development can be determined. Several experiments
have linked regular exercise with increased health of endothelial (increased NO production) and smooth muscle cells (increased relaxation).⁷

Figure 1: NO in the Endothelial and Smooth Muscle Cell
Created by Kerry Clifford and adapted from: http://basic-clinicalpharmacology.net/chapter%2019_%20nitric%20oxide_files/image010.gif

**Exercise Benefits to Women**

Currently, only 26% of women in the United States follow the current exercise guidelines set by the American College of Sports Medicine.⁹ It is well established that women who exercise on a regular basis (30 minutes on most days of the week) have a
decreased risk of developing cardiovascular disease, obesity, diabetes and hypertension.\textsuperscript{10} It is well recognized that acute exercise induces multiple changes to the cardiovascular system, such as increasing heart rate, oxygen delivery and blood flow.\textsuperscript{9} It is the accumulation of these acute bouts of exercise over time that is thought to lead to reductions in cardiovascular disease through the modification of traditional risk factors for heart disease. However, it has recently been reported that the positive effects of exercise on traditional risk factors can only account for 60\% of the cardiovascular disease risk reduction that result from exercise.\textsuperscript{7} This has led many to speculate that changes in hemodynamics during exercise account for the other 40\% in cardiovascular risk reduction.\textsuperscript{3,7} In addition, chronic exercise has been determined to alter vascular function due to increased blood flow and shear stress.\textsuperscript{7} Exercise induced shear stress placed upon the arterial wall has been posed as a possible mechanism of the alterations in vasculature following an exercise training routine.\textsuperscript{7} In addition, increase in nitric oxide release during exercise has been documented as the specific mechanism of vascular health improvements.\textsuperscript{6} The specific mechanisms of alterations in vasculature and risk reductions from exercise are still to be elucidated. Regular exercise decreases the occurrence of cardiovascular events and additional vascular alterations following training are still to be elucidated.

\textbf{Developmental Origins of Disease}

It is well known that diet, exercise, genetics and other lifestyle factors play a role in the development of adulthood disease. However, it is also known that the risk of
disease development begins in the intrauterine environment and adulthood disease may originate during critical periods of fetal development. The importance of the intrauterine environment is likely due to fetal programming and the fetal gene structure. Since several phenotypes occur from less genotypes, scientists suggest that events throughout gestation alter the fetal gene expression. Several studies suggest that negative environments placed upon the fetus during critical stages of development result in long-term alterations of the fetal gene structure. Research has observed significant differences in offspring exposed to maternal smoking, alcohol, poor nutrition and stress when compared to control groups.

_Barker Hypothesis_

The first series of epidemiological studies that detailed fetal programming and the importance of maternal environment were performed by David Barker and colleagues. They examined previous birth and death records from the Netherlands during the Dutch famine. The famine induced an undernutritional model to mothers and offspring. A clear relationship was noted between the offspring of mothers that experienced famine throughout pregnancy and offspring disease later in life. Babies exposed to the famine were typically of low birth weight (<2500g) and were correlated with increased risks of cardiovascular disease in adulthood. Barker concluded that permanent adaptations during fetal development increased the risk of disease onset in adulthood. These early epidemiological studies lead to the hypothesis of the developmental origins of adult disease, also known as the Barker Hypothesis.
It is clear that a growing fetus depends upon its mother’s reserve and dietary intake for nutrients and oxygen to grow. Since the American diet has become less nutritious and of higher energy, maternal nutrition during pregnancy has become a larger focus of study. In addition, the idea that pregnant women are eating for two has led to more high birth weight babies (above 4000 g) than ever before. Many women feel that it is better to eat excess calories, than insufficient calories. However, science continues to prove that both overnutrition and undernutrition both are detrimental to a growing fetus. Studies have significantly proven that offspring birth weight represents a “U-shaped” relationship with adult fat mass, obesity and disease onset. Interestingly, offspring of low birth weight and high birth weight demonstrate similar susceptibility to cardiovascular disease later in life.

Overnutrition

Maternal hypernutritional status can be defined as excess calorie intake compared to energy expenditure. Maternal overnutrition has been linked with higher birth weight offspring and disease later in life. Although development of obesity is largely associated with genetics, one study proved that maternal obesity is more highly associated with offspring obesity when compared to paternal obesity. The increased relationship between fetal birth weight and maternal weight lends support to the developmental origins hypothesis that disease begins in the intrauterine environment. Being that over 50% of women of reproductive age are either overweight, or obese, future offspring are at increased risk of developing disease without a proper intervention.
Hyper nutritional status not only puts offspring at a higher risk of becoming obese, but also increases risks of diabetes, insulin insensitivity, hypertension, cardiovascular disease and metabolic syndrome in adulthood.\textsuperscript{5,12} Female offspring of mothers exposed to a high-fat diet during gestation have also been reported to have significantly higher adiposity compared to lean mass.\textsuperscript{5,12,13} It has been proposed that hypernutrition programs altered cardiovascular system pathways in offspring which plays a large role in adulthood atherosclerotic disease development.\textsuperscript{5} Interestingly, one study reported that a hypercaloric fetal environment lead to endothelial dysfunction in adult offspring.\textsuperscript{5} This data highlights the fetal response to a hypercaloric nutritional environment as a means of preparing for adulthood in a similar hypernutritional environment.\textsuperscript{5} The preparation for adulthood occurs by alterations in the fetal gene structure. This research further supports the importance of the intrauterine environment and fetal origins of adulthood disease.

\textit{Undernutrition}

Maternal undernutrition during pregnancy is not as common as overnutrition in the United States. However, maternal protein restriction is the most common type of malnourishment in the United States.\textsuperscript{6} A suboptimal nutritional environment results from a negative maternal energy balance, resulting in forced adaptations to a negative intrauterine environment.\textsuperscript{6}

Several other related diseases such as cardiovascular disease, diabetes and metabolic syndrome are associated with a poor nutritional environment during
pregnancy. One study established significant data to support that birth weights below 2500g are at an increased risk of developing type 2 diabetes and other related disease later in life. A possible mechanism underlying maternal malnutrition during pregnancy relates to reduced nutrient delivery to the placenta, in turn decreasing the fetal glucose uptake and programming disease later in life.

Specifically, caloric restriction throughout pregnancy has been reported to influence the cardiovascular health in offspring. Negative intrauterine environments can increase adult onset of high blood pressure, dyslipidemia and glucose intolerance. Maternal caloric restriction of 50% increased fetal body fat as adults and programmed high triglycerides and less lean mass. Vascular function experiments have also been utilized to study offspring exposed to undernutrition. It is established that endothelial dysfunction is associated with offspring of low birth weight. A mechanism of dysfunction can be attributed to rapid catch up growth after birth in children of low birth weight, and has often been hypothesized to play a role in poor vascular health. In addition, a study of 9-year-old children proved that low birth weight increased carotid artery stiffness, a precursor of hypertension and other related cardiovascular diseases. Another study reported that children of mothers who had a negative energy balance had an increased intima-medial thickness (IMT). Intima-medial Thickness is commonly used in research to predict susceptibility to cardiovascular disease later in life.

The impact of undernutrition during pregnancy on cardiovascular outcomes of offspring has also utilized rodent and pig models. Research by Yates et. al. demonstrated the effects of maternal undernutrition on the atherosclerotic susceptibility in rodent offspring. After the birth of the offspring, they were fed either a standard
chow diet or an atherogenic diet, which causes large increases in circulating cholesterol. Offspring from Low-protein fed mothers led to higher incidence of dyslipidaemia (p<.001). In addition to dyslipidaemia, offspring had more severe atherosclerotic lesions in aortic arches (p<.001).

Animal models have also been used in in-vitro wire myography experiments in order to assess vascular function of offspring. One particular study exposed offspring to nutrient restriction and hypoxia during gestation. Maternal caloric restriction and hypoxia did affect the offspring response to an exogenous NO donor, sodium nitroprusside (SNP). Previous studies have demonstrated a wide range of results when given an exogenous NO donor such as sodium nitroprusside. However, this particular data set suggests that there is decreased availability of NO in the control group, instead of an actual decrease in smooth muscle sensitivity. Further research concluding the effects of the fetal environment on the offspring smooth muscle sensitivity are needed in order to determine further mechanisms of disease.

**Maternal Exercise**

Many negative intrauterine environments have previously been researched in coordination with vascular health and cardiovascular disease. However, exercise as a positive intrauterine environment has more recently been studied. Several studies have tested this hypothesis utilizing different animal models. Scientists demonstrate that maternal exercise during pregnancy may be the earliest intervention to improve
cardiovascular health of offspring.\textsuperscript{10} Finding the exact mechanism for disease, specifically atherosclerosis, would benefit the future population.\textsuperscript{4,9,10,15}

Currently, The American College of Obstetricians and Gynecologists (ACOG) and The American College of Sports Medicine (ACSM) recommend that pregnant women that are free of medical complications during pregnancy engage in 30 minutes or more of moderate physical activity on most, if not all, days of the week.\textsuperscript{15} Although, recent studies have reported that only 15\% of pregnant women follow these recommendations.\textsuperscript{15,16} In addition, it has been reported that 69\% of women are advised to restrict their activity during gestation.\textsuperscript{16} It is not uncommon for women to feel that vigorous exercise will harm their child’s development and that rest is more important for a developing fetus.\textsuperscript{16} Although there is limited data to suggest that maternal exercise has positive effects on an offspring’s susceptibility to atherosclerosis, several pieces of evidence separately support this notion. There are several benefits of exercise to the non-pregnant subject and studies have demonstrated that pregnant women benefit the same way as non-pregnant women.\textsuperscript{9}

\textit{Maternal Benefits}

Data suggests that nearly all women experiencing a normal pregnancy can benefit from following an exercise routine.\textsuperscript{17} Several cardiovascular changes occur at the onset of pregnancy.\textsuperscript{9} Pregnant women experience increased resting heart rate, sub maximal heart rate and increased blood volume.\textsuperscript{9} Blood pressure and blood glucose throughout a healthy pregnancy should remain at normal levels, however this is not always the case.\textsuperscript{9} Although changes occur, women who continue pre-pregnancy exercise routines
experience less trouble with preeclampsia, proteinuria and gestational diabetes.\textsuperscript{17} In addition, maternal exercise can treat or possibly prevent gestational diabetes, which occurs in 17% of obese women (currently 35% of pregnant women are obese).\textsuperscript{17,18} Regular maternal exercise has also been shown to have positive effects, such as decreased pain and discomfort, reduced depression, shorter labor, fewer complications and a faster recovery after delivery.\textsuperscript{10} While there are many uncovered benefits to the mother, fetal outcomes in response to maternal exercise are the focus of this research.

\textit{Fetal Outcomes}

Exercise has many positive effects on a developing fetus and more benefits are still to be elucidated.\textsuperscript{9} Although, there are few adverse outcomes that relate to the connotations of maternal exercise.\textsuperscript{9} Many women feel an increased body temperature during exercise will harm the fetus. However, studies demonstrate that moderate intensity activities in normal environments will not harm the fetal body temperature.\textsuperscript{9} Research suggests there is no abnormal fetal growth in offspring of exercised mothers with adequate calorie intakes.\textsuperscript{9}

There are several positive fetal outcomes in response to maternal exercise throughout gestation. The main fetal response is a cardiovascular adaptation that results in a lower overall resting heart rate.\textsuperscript{10} In addition, improved stress tolerance and advanced neurobehavior has been documented in fetus’ whose mothers followed a regular exercise routine.\textsuperscript{9} Many benefits have been observed in offspring exposed to maternal exercise,
however this research focuses upon the cardiovascular benefits to offspring exposed to maternal exercise.

A study completed by Gale et. al. measured intima-media thicknesses in children (n=216). A larger intima-media thickness is often associated with increased susceptibility to Cardiovascular Disease later in life. Offspring who were exposed to strenuous maternal exercise had a non-significant decrease in carotid intima-media thickness (p-value=.084). Even though the data was not significant, a marked decreased in IMT thickness must be noticed in children of mother’s that exercised. This suggests that offspring exposed to maternal exercise may be at a decreased risk of developing disease later in life.

Fetal heart rate (HR) and heart rate variability (HRV) have also been reported to be affected by maternal exercise during pregnancy. One study determined that fetal HR was significantly lower (p=<.0006) in the fetus’ of exercised compared to sedentary mothers. A higher Fetal HRV is typically seen in trained subjects and indicates increased cardiovascular health.

Several benefits to a developing fetus occur from maternal exercise; however not enough studies have been completed to date. Further research needs to be done in order to determine further benefits of maternal exercise. However, the supporting data demonstrates that nutrition and exercise interventions can reduce disease risk in most populations. The literature is lacking that investigates the actual effects of maternal exercise on endothelial and smooth muscle cell function of offspring. This research was designed to investigate the effects of maternal exercise on endothelial and vascular
smooth muscle cell function in offspring. We hypothesized that there would be no
change in smooth muscle cell function in offspring.
METHODS

Animals

Due to the similarity between swine and human cardiovascular systems and atherosclerotic disease susceptibility, crossbred Swine were used for this study.\textsuperscript{22,25} Six-month old primiparous gilts were used for this intervention. For one week the gilts were acclimated to the treadmill and humans. The ovarian cycles of the gilts were controlled by an active progestin diet. Gilts were artificially inseminated and randomized into two groups: exercise (n=8) and sedentary (n=8). Both intervention groups were fed a standard chow diet of 3.25kg per day. After four weeks, pregnancy was confirmed and non-pregnant gilts were excluded from the study. Gilts were excluded if they were not pregnant or if they were not compliant for the entire protocol. Four exercise and four sedentary gilts remained in the study for 16 weeks of protocol. The remaining gilts were individually housed in a large animal facility at Purdue University. All protocol and handling of the animals was approved by the Purdue Animal Care and Use Committee (PACUC).
Image 1: Exercise gilt during intervention

Image 2: Sedentary gilt in cage during intervention
Exercise Protocols

The exercised gilts were acclimated to treadmill through the use of food. The sedentary gilts were placed in a cage directly next to the treadmill to provide a similar environmental stimulus. The gilts were exercised on a specialized pony treadmill (Mini Sport Pony Treadmill, Trilogy) 5 times per week for 20 to 45 minutes per day. For 15 weeks throughout gestation, the exercise intensity ranged from 65-85% maximal heart rate. The heart rate data was assessed through the use of a heart rate monitor (Polar S810, Polar Electro Inc., Lake Success, NY). The methods of positive reinforcements were standardized for both groups. Animals stopped exercising one week prior to birth. Both the exercise and sedentary gilts were weighed on a weekly basis and results were recorded. A livestock scale was used for both the pregnant and exercise groups.
Offspring and Tissue Collection

One month after birth, the offspring were weaned from their mothers. Following weaning, all offspring were group housed and fed ad libitum of a standard pig chow diet. Offspring were sacrificed at 3, 5 and 9 months of age. A total of 20 offspring from exercise mothers and 22 offspring of sedentary mothers were sacrificed at each time point. Two offspring (1 male and 1 female) were randomly selected each day for assessment of vascular function in response to maternal exercise. The offspring were euthanized and the femoral arteries were harvested. From each offspring, a 10 cm section of the right femoral artery was removed and stored in a physiological Krebs solution to preserve function. The sex of the offspring was matched during in-vitro experiments. Measurements of offspring length and weight were taken immediately following birth and again at sacrifice.

In-Vitro

Following the dissection, the arteries were cleaned of fat and connective tissue. Vessels were cut into three sections (~3mm). The arteries were measured for length and diameters on a stereomicroscope (PZMIII, World Precision Instruments, Sarasota, FL, USA) along with Image J software (NIH, Bethesda, MD). Six arterial segments were hung on wire myograph (Myobath II, World Precision Instruments, Sarasota, FL) in an alternating order between sedentary and exercise offspring. Each arterial segment was immersed in a 20 mL water bath of Krebs Solution at 37°C, bubbled with 95% O₂ and 5% CO₂ mixture. The resting tension was determined using previous data and the arteries were pre-constricted using prostaglandin (PGF₂) (30 µM)\(^{21}\). Sodium nitroprusside (SNP; \(10^{-10}–10^{-4}\))
M) was used as an endothelium-independent vasodilator to determine specific function of the vascular smooth muscle cells. The results of relaxation were then transmitted through a force transducer for analysis.

Image 3: In-vitro wire myography setup
Statistics

Dose response curves to sodium nitroprusside was expressed using ANOVA. The ANOVA model used the intervention for each gilt to exclude correlation between offspring from the same gilt. The SNP data were demonstrated as a percent relaxation of the PGF2 and baseline tensions. The P-Value used for these experiments was set at P<0.05. The data were reported as a mean and standard error for each outcome. For this report, data from the 3, 5 and 9 month offspring were analyzed.
RESULTS

Sow Training and Characteristics

In the exercised animals, maternal exercise averaged 39.5 ±0.9 minutes per session throughout 15 weeks of the protocol (Figure 3). Maternal weight gain was significantly lower in exercised (70.5 ±4.8kg) sows versus sedentary (83.7 ±3.8kg) sows (Figure 4). The resting heart rates were also significantly lower in the exercise trained (95 ±4 bpm) versus the sedentary group (107 ±3 bpm) after 15 weeks of gestation (p=0.047) (Figure 5).
Figure 3: Gilt exercise duration throughout gestation ranged from 25 to 45 minutes per session.
Figure 4: Sow weight gain throughout gestation for exercise and sedentary pigs

*(Significant, p<0.05)*
Figure 5: Resting heart rates in exercise and sedentary gilts at 15 weeks of gestation *(significant, p<0.05).*
Litter Characteristics

There were no significant differences in litter sizes between the exercise and sedentary groups. Piglet weights, lengths and girths were also not significantly different between offspring of exercise and sedentary mothers (Table 1). The sacrifice weights were also taken at 3, 5 and 9 months and were not significantly different between intervention groups (Table 1).

Table 1: Offspring Characteristics of 3, 5 and 9 Month

<table>
<thead>
<tr>
<th></th>
<th>3 Month</th>
<th>5 Month</th>
<th>9 Month</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sedentary (n=8)</td>
<td>Exercised (n=7)</td>
<td>Sedentary (n=7)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.48 ±0.14</td>
<td>1.36 ±0.11</td>
<td>1.57 ±0.10</td>
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<tr>
<td>Birth length (cm)</td>
<td>35.88 ±0.99</td>
<td>34.13 ±1.1</td>
<td>35.43 ±1.4</td>
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<tr>
<td>Birth girth (cm)</td>
<td>24.6 ±0.68</td>
<td>23.3 ±0.86</td>
<td>25.1 ±0.55</td>
</tr>
<tr>
<td>Sacrifice Wt. (kg)</td>
<td>49.2 ±2.5</td>
<td>47.7 ±1.87</td>
<td>122.5 ±4.9</td>
</tr>
</tbody>
</table>

Values are represented as means and ± standard errors.
Vessel Characteristics

There were no significant differences in the vessel characteristics (artery lengths, thickness, diameters) between the exercise and sedentary groups (Table 2). The SNP resting tension (g) was also not significantly different between groups (Table 2). However, the PGF induced tension was significantly lower in the exercise versus the sedentary groups at 3 and 9 months of age (Table 2). At 5 months of age were was not a significant difference between the PGF induced tension (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>3 Month</th>
<th>5 Month</th>
<th>9 Month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sedentary (n=8)</td>
<td>Exercised (n=7)</td>
<td>Sedentary (n=7)</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>3.02 ±0.22</td>
<td>2.70 ±0.10</td>
<td>3.17 ±0.14</td>
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<tr>
<td>Outer diameter (mm)</td>
<td>3.91 ±0.08</td>
<td>3.71 ±0.12</td>
<td>4.83 ±0.20</td>
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<tr>
<td>Inner diameter (mm)</td>
<td>2.54 ±0.15</td>
<td>2.39 ±0.13</td>
<td>3.01 ±0.26</td>
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<tr>
<td>Wall thickness (mm)</td>
<td>0.69 ±0.04</td>
<td>0.66 ±0.05</td>
<td>0.90 ±0.05</td>
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<tr>
<td>SNP Resting tension (g)</td>
<td>7.57 ±0.36</td>
<td>7.83 ±0.40</td>
<td>7.58 ±2.60</td>
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<tr>
<td>PGF tension (g)</td>
<td>37.3±1.9*</td>
<td>30.0 ±0.77*</td>
<td>51.8 ±3.1</td>
</tr>
</tbody>
</table>

Table 2: Vessel Characteristics of 3, 5 and 9 Month
Values are represented as means and ± standard errors. * = significantly different
Dose Response

At 3, 5 and 9 months, both the exercise and sedentary groups illustrated an increased response to increasing doses of sodium nitroprusside (Figure 6-8). Sedentary offspring had a significantly greater relaxation response at both 3 and 9 months of age when compared to offspring from exercised sows (*, p<0.05). There were no significant differences in femoral artery sodium nitroprusside response between sedentary and exercised offspring at 5 months of age (Figure 7).
Figure 6: Relaxation responses to sodium nitroprusside for 3 month olds

*Significantly different (p<0.05)
Figure 7: Relaxation responses to sodium nitroprusside for 5 month olds
Figure 8: Relaxation responses to sodium nitroprusside for 9 month olds

*Significantly different (p<0.05)
DISCUSSION

The purpose of this investigation was to determine the effects of maternal exercise on the vascular smooth muscle cell function of offspring. The current findings suggest that vascular smooth muscle responsiveness to sodium nitroprusside is reduced in the offspring of exercised mothers compared to the offspring of sedentary sows at 3 and 9 months of age (Figure 6 and 8). There could be age related alterations at 5 months of age mediating the non-significant results. However, there was a trend in the 5 month data in the exercise group in which the exercise group had reduced function. It is still unclear the reason behind the significant differences at 3 and 9 months of age and not at 5 months of age. These findings are not consistent with our hypothesis that maternal exercise would have no effect on vascular smooth muscle cell function of offspring.

Several studies have concluded that offspring are largely affected by the intrauterine environment.\(^5\) While most studies have focused on maternal nutrition, there is evidence to suggest that exercise during pregnancy may be the earliest intervention for cardiovascular disease prevention in future offspring.\(^4\) Previous studies of maternal exercise during pregnancy have reported positive effects on the fetal heart rate, heart rate variability and carotid intima-media thickness in offspring.\(^4,10\) It has also recently been reported that maternal exercise during pregnancy increases endothelial function in swine offspring at 48 hours after birth and has no impact on endothelium-independent function.\(^23\) Our current findings of decreased endothelium-independent relaxation in offspring from exercise-trained mothers is contrary to the previously reported results at 48 hours after birth. The mechanisms behind the differences in the results are still unclear. Two potential mechanisms behind the differences in vascular smooth muscle cell function at
48 hours and 3-9 months of age may be ageing or differences in dietary intake after weaning. The offspring were housed together and fed *ad libitum* after weaning which may have contributed to the differences. Future studies will need to elucidate the causes of the differences in the vascular smooth muscle cell results between the two studies.

One potential mechanism underlying the reduced vascular smooth muscle cell function in the offspring of exercised mothers may be linked with decreased nutrient delivery to the fetus during exercise. It is well established that caloric restriction during pregnancy yields a smaller litter and body size in offspring. These reductions in birth weight are associated with decreased vascular function in offspring. Consistent with previous studies, the sow weight gain was significantly lower in the exercise versus the sedentary mothers (Figure 4). These differences in weight can be attributed to the increased utilization of energy during exercise. An important finding in the current study is that the maternal weight gain was significantly different, but did not alter the offspring body weight and litter sizes. Therefore, reduced nutrient delivery to the fetus during maternal exercise cannot be the cause of the altered vascular smooth muscle cell responses in the exercised offspring.

Another potential mechanism behind the reduced vascular smooth muscle response in the exercised group may correlate with maternal stress. Previous studies have reported that maternal stress directly relates to reduced vascular function in offspring. In the current study there were no direct measurements of maternal stress. However, throughout the protocol the animals voluntarily exited cages and loaded onto the treadmill. In addition, the gilts did not show signs of stress during the exercise intervention (vocalization and bar chewing). Therefore, maternal stress during the exercise intervention is not a likely
mechanism underlying the reductions in vascular smooth muscle cell response in exercised offspring.

The results of the current study demonstrated a reduced vascular smooth muscle function in the exercised group. These results could be interpreted that exercise during pregnancy negatively affects the vascular health of the offspring. Alternatively, these findings could be a result of a positive adaptive response in offspring exposed to maternal exercise. A scenario that could account for these positive adaptive responses could be caused by epigenetic modifications in the offspring, which is driven by changes in hemodynamics during maternal exercise. It is well established that fetal heart rate increases during maternal exercise, which in turn leads to changes in fetal hemodynamics. Alterations in hemodynamics are known to produce epigenetic modifications that influence gene expression. One potential gene that has been reported to be affected by hemodynamic changes is eNOS.

Elevations in eNOS protein result in increased NO production. Elevated NO bioavailability causes the vascular smooth muscle to relax through the guanylate cyclase pathway. One can speculate that increased NO bioavailability will lead to the down regulation of guanylate cyclase (Figure 9). However, in this study where exogenous NO was given, (sodium nitroprusside), a down regulation of guanylate cyclase would produce a reduced relaxation of the vascular smooth muscle cells. Alternatively, in sedentary animals reduced NO bioavailability would lead to the up regulation of guanylate cyclase and produce a greater vascular smooth muscle cell response to exogenous NO (Figure 9). Therefore, in the current study, epigenetic modifications and the down regulation of
gaunylate cyclase pathway could be the mechanism underlying the blunted relaxation in the offspring exposed to maternal exercise.

Figure 9: The effect of endogenous NO in exercise and sedentary offspring and the effects of exogenous NO in the exercise and sedentary offspring. Image made by Kerry Clifford.

In conclusion, the current findings demonstrate a reduction in the vascular smooth muscle cell function in offspring of exercised mothers at 3 and 9 months after birth. It is currently unclear if this adaptive mechanism will decrease or increase the atherosclerotic susceptibility of offspring. Future studies should investigate the underlying causes of
these alterations in the vascular smooth muscle cell function to elucidate the significance of these findings.
LIST OF REFERENCES

## Appendix

Data used for the graphs.

### Exercise speed

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