AEROBIC FITNESS AND THE RENIN ANGIOTENSIN SYSTEM IN ADOLESCENTS BORN PRETERM WITH VERY LOW BIRTH WEIGHT

BY

CHRISTOPHER R. KAISER

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Approved By:

Patricia A. Nixon, PhD, Advisor

Peter Brubaker, PhD, Chair

Lisa K. Washburn, MD
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INTRODUCTION

Preterm (PT) birth is defined as being born at less than 37 weeks gestation. Although the incidence of PT birth has decreased in recent years, as of 2014, 9.6% infants were born PT.\(^1\) Additionally, 1.4% of all live births in the U.S. are very low birth weight (VLBW < 1500g).\(^2\) PT infants are at a higher risk of infant death compared to healthy term born peers, with PT birth accounting for 1/3 of infant mortality.\(^1\)\(^,\)\(^3\) Survivors of PT birth face a number of short and long-term consequences including respiratory, neurodevelopmental, and cardiovascular complications, in addition to increased mortality.\(^3\)

Adults born PT have been shown to have higher incidence of hypertension (HTN) and mortality due to cardiovascular disease (CVD) risk when compared with healthy term born peers.\(^4\)\(^,\)\(^5\) This relationship may be partly attributable to the fetal origins of adult disease (FOAD) hypothesis, which proposes that adverse events during key developmental windows alter organ system structure and function that promotes survival in the short-term, but may be detrimental in the long-term, leading to higher risk for developing chronic diseases such as hypertension, cardiovascular disease, and diabetes later in life.\(^6\)\(^,\)\(^7\)

The kidney is one organ system that may be affected, and is especially important in terms of blood pressure (BP) regulation. Nephrogenesis begins by 9 weeks gestation, however kidney formation is not complete until around 32-36 weeks.\(^8\) Both animal and human studies indicate that PT birth is associated with reduced nephron number, which may lead to hypertension.\(^8\)\(^,\)\(^9\) There is growing
evidence that the renin angiotensin system (RAS), another key component of BP control, may also be affected by both PT birth, as well as kidney development.\textsuperscript{10–13} In RAS, Angiotensin II (Ang II) acts as a vasoconstrictor, ultimately raising BP, whereas Angiotensin 1-7 (Ang 1-7) acts as a vasodilator and lowers BP.\textsuperscript{14,15} High levels of Ang II is associated with higher blood pressure, and chronic diseases such as CVD and diabetes; on the other hand Ang 1-7 has been shown to have cardio protective effects.\textsuperscript{16} When compared with healthy term born peers, adults born PT and/or VLBW have been shown to have elevated levels of plasma Ang II, in addition to elevated levels of angiotensin converting enzyme (ACE; the enzyme that converts ANGI to AngII), ultimately implicating RAS as having a role in developing HTN within this population.\textsuperscript{10,11,13}

Preterm birth has also been associated with reduced cardiorespiratory fitness as well as participation in physical activity (PA).\textsuperscript{17,18} It is well established that individuals who participate in more PA, or have higher aerobic fitness, are at lower risk of developing hypertension and other cardio metabolic diseases.\textsuperscript{19–22} Consequently, lower PA and aerobic fitness may put this predisposed population at even greater risk for developing HTN and other diseases.\textsuperscript{17,23–25}

Little research has been done examining the influence of PA and fitness on RAS, with no studies examining these relationships in PT populations. A meta-analysis examining the effects of exercise on RAS found lower plasma renin activity post aerobic training.\textsuperscript{26} Additionally, one animal model demonstrated that rats exposed to a 16 week PA program had lower levels of ACE, Ang II, and BP when compared to rats that did not participate in PA.\textsuperscript{27} If this same relationship exists in
humans, then adults born PT who participate in PA and have higher cardio respiratory fitness (CRF) may have more favorable RAS, and subsequently lower BP and HTN risk.

In summary, there is some evidence that being born PT and/or VLBW is associated with higher ACE and Ang II levels\textsuperscript{10,11,13} which in turn has been associated with hypertension and other cardiometabolic diseases. There is evidence that PT/VLBW persons have lower PA and fitness, which is also associated with higher BP and other chronic diseases.\textsuperscript{19,20,23,24} Lastly, there is evidence in animal models that PA may decrease the ACE/Ang II levels.\textsuperscript{27} However, to date no study has examined the influence of PT birth on RAS and the role that fitness and PA may have on its association.

Therefore, the aims of this study are to examine RAS in PT/VLBW adolescents compared to their term-born peers, and the potential mediating influence of PA and fitness on the association between PT birth and RAS. Understanding this relationship may help to determine preventative or therapeutic strategies for this at risk population.
REVIEW OF LITERATURE

Epidemiology

Although the incidence of PT birth has declined in recent years, it still remains a major public health concern. According to the Center for Disease Control and Prevention, PT birth accounts for roughly 9.6% of all live births in the U.S.\(^1,2\). Although PT birth in and of itself acts as the greatest contributor to infant death, accounting for 1/3 of infant mortality, survivors of PT birth also face a number of short and long-term consequences\(^1,2\).

In the short term, infants born prematurely are at greater risk of difficulties including respiratory dysfunction, leading to the dependence on mechanical ventilation and supplemental oxygen post birth. Cardiovascular complications include patent ductus arteriosis, and unstable BP. PT infants also have increased risk of necrotizing enterocolitis, and brain hemorrhage. Their immune system and blood glucose regulation may also be impaired\(^3\). As a result of preterm birth, infants are subjected to prolonged intensive medical care post birth, which not only causes further stress on the infant, but places stress and economic burden on families\(^3\).

Many of the short-term complications may persist placing survivors of preterm birth are at risk for neurodevelopmental conditions such a cerebral palsy, impaired learning, behavioral disorders, and impaired neuromotor development in childhood, as well as chronic conditions such as cardiovascular disease (CVD), asthma, obesity, diabetes and HTN in adulthood.\(^3,17\) It has also been reported that
survivors of PT birth report lower levels of PA, and have lower levels of aerobic fitness which may further increase their risk for chronic diseases.28,29
Fetal Origins of Adult Disease (FOAD)

It has been proposed that some of the long-term health consequences faced by survivors of PT birth may be accountable for by FOAD. Barker originally proposed the FOAD hypothesis which states that adverse events taking place during key windows of organ system development may impact health later in life. In Barker’s hypothesis, intrauterine growth restriction (IUGR) was discussed as one of the adverse events leading to adult diseases, specifically diseases related to BP, cholesterol, metabolism, insulin response to glucose, as well as endocrine and immune function.

Of particular interest to this thesis is Barker's discussion of chronic diseases such as HTN, and it's prevalence in populations born with low birth weight (LBW< 2500g). In a systematic review, Barker included 34 different studies where BP measurements were taken at ages ranging from 3 years old – 71 years old. The studies included represented varying age groups and countries, and included over 66,000 participants. A majority of the 34 studies report an inverse relationship between birth weight and BP, with Barker concluding that a 1kg increase in birth weight was associated with a 3.5 mmHg decrease in SBP. While a BP decrease of 3.5 mmHg may not seem clinically significant, Barker goes on to explain that there are large differences in mean population values, and that by lowering the mean systolic BP value in a population by 10 mmHg, the attributable mortality rate would drop by roughly 30%. It is important to note that while all of the studies examining
this relationship in adults reported the same inverse linear relationship, studies including adolescents reported conflicting results. Barker explains that these findings in adolescents may be attributable to naturally fluctuating blood pressure tracking in adolescents due to growth spurts, and that higher BP may not appear until adulthood.

Calkins and Devaskar elaborate on Barker’s initial report in their review examining the long term health outcomes associated with being born PT and/or LBW. The authors report that a number of historical studies such as the Helsinki and Hertfordshire studies, comprising over 20,000 subjects, have found that poor fetal growth is strongly correlated with coronary artery disease (CAD), insulin resistance, and HTN in adulthood. Furthermore, LBW was associated with an increased death rate from CAD. Additional studies have demonstrated that LBW is also inversely correlated with insulin sensitivity. In one study examining the birth characteristics of children who developed type II diabetes (T2DM), it was found that the odds ratio for developing T2DM was 1.38 for every kg decrease in birth weight.

**Birth Weight and Muscular Development**

Skeletal muscle mass is a critical component of metabolic health; accounting for 80% of insulin-stimulated glucose uptake, improving insulin sensitivity, and preventing the onset of type II diabetes. A number of studies have shown that being born with low birth weight as the result of IUGR may compromise skeletal muscle mass. Furthermore, there is evidence that birth weight is closely
associated with fat free mass, which influences aerobic fitness. Ultimately, having less muscle mass as the result of low birth weight may decrease aerobic fitness and increase cardiometabolic disease risk.

Animal studies have demonstrated that myogenesis, or muscle cell development, is nearly complete at birth, and that postnatal catch up growth is comprised of myofiber hypertrophy, not an increase in myofiber number (hyperplasia). Animals that were born with IUGR had reduced muscle mass, and a 33% decrease in skeletal muscle precursor cells, indicating insults during fetal development compromise skeletal muscle mass. Singhal et al. has demonstrated the same relationship in humans, and reported that LBW was significantly associated with lower lean body mass when measured via DXA in adolescents. This relationship was independent of age, sex, pubertal stage, physical activity, and height.

Further evidence exists suggesting a link between LBW and muscular developmental programming and metabolic function. Hansen et al. collected muscle biopsies from 23 formerly LBW 16 year old men and 16 age matched healthy controls. Glucose uptake, glucose transport, insulin signaling, markers of muscle cell maturity, and mitochondrial gene expression were measured. Hansen found that formerly LBW individuals had reduced glucose uptake, and decrease glucose transporter levels compared with NBW controls. Additionally, the LBW group had decreased myogenic differentiation markers, suggesting impaired muscle cell development. The authors conclude that formerly LBW individuals had both
transcriptional and metabolic alterations effecting the muscle cells, and that these defects were likely the result of developmental programming.\(^{39}\)

Ridgway et al.\(^ {36}\) has shown that the lower muscle mass associated with being born with lower birth weight contributes to lower aerobic fitness. The study was conducted on cohort of 2749 adolescents from the European Youth Heart Study. Measurements were taken to determine body composition and aerobic fitness. The authors found that birth weight was significantly associated with fat free mass, which was associated with aerobic fitness, and that lower birth weight was also associated with lower fitness.

Additional research suggests that LBW individuals have altered skeletal muscle fiber type as a result of fetal programming.\(^ {40}\) Jensen et al. analyzed biopsies of vastus lateralis muscle from 8 LBW and 12 NBW 19 year old men, and found that LBW was associated with an increased proportion of type IIx fibers at the expense of a decrease in type IIa muscle fibers.\(^ {40}\) Because type IIx fiber rely heavily on anaerobic metabolism, while type IIa fibers have a high capacity for both aerobic and anaerobic metabolism, an increase in type IIx and a decrease in type IIa muscle fibers may affect aerobic capacity.\(^ {41}\) Furthermore, Brons et al. found that individuals born LBW had decreased oxidative phosphorylation gene expression in skeletal muscle, which may further exacerbate impaired aerobic capacity;\(^ {42}\) however a healthy diet was found to attenuate this relationship.
Birth Weight, Prematurity, and Cardiometabolic Disease

In addition to LBW and IUGR, being born PT is another well-documented insult that is correlated with adult disease. In one systematic review and meta-analysis, Jong et al. analyzed 10 studies composed of 1342 PT and/or very low birth weight (VLBW <1500 g) participants, representing 8 countries. Of the 10 studies included in the analysis, the mean gestational age was 30.2 weeks, the mean birth weight was 1280g, and the mean age at BP measurement was 17.8 years. They found that based on their most adjusted pooled estimate of SBP difference between PT/VLBW and term controls, individuals born PT/VLBW had SBP 2.5 mmHg higher than term born peers (95% CI 1.7-3.3 mmHg). Additionally, they found that when looking at only the five highest quality studies, the difference in SBP increased to 3.8 mmHg (95% CI 2.6-5.0 mmHg). Jong et al. concluded that PT/VLBW infants have higher SBP in adulthood compared to term born peers.

There are numerous studies examining the relationship between birth weight and the risk of developing diabetes. In one meta-analysis including fourteen studies, and a collective sample of 132,180 participants, LBW was found to be associated with an increased risk of developing type 2 diabetes mellitus (T2DM), with an odds ratio of 1.32. Additionally, when normal birth weight was used as the reference category, the odds ratio increased to 1.47.

Lastly, individuals born LBW have also been shown to be at higher risk for developing CVD. In a meta-analysis including twenty-two studies assessing the associations between birth weight and all-cause mortality, a strong inverse
association existed between birth weight and mortality due to CVD. The authors found that there was a 12% reduced risk of CVD mortality for every kg increase in birth weight.

To summarize, the FOAD hypothesis may have serious implications for survivors of PT birth and/or LBW, especially in terms of increased risk for HTN and the development of other cardiometabolic diseases. Although there have been numerous studies conducted on the relationship between being born PT/LBW and subsequent BP, these studies report conflicting results. While the majority of studies suggest an inverse relationship between BW and SBP, it has been suggested that this relationship may not be evident in adolescence.
**Renin Angiotensin System (RAS)**

One system common to hypertension, diabetes and CVD is the RAS.\(^{16}\) The RAS has long been understood to play a crucial role in blood pressure regulation.\(^{16}\) Furthermore, recent studies have implicated an imbalanced RAS as a precursor to chronic diseases such as CVD, diabetes, and HTN.\(^{15,16,54}\)

While many studies have demonstrated increased CVD risk in PT/LBW individuals, few studies have reported on the underlying pathophysiological mechanisms behind this relationship.\(^{6,11}\) One explanation is that individuals born PT/LBW have an altered RAS, an important hormone system involved in BP regulation.\(^{11}\)

*Figure 1: The Renin Angiotensin System*  
Source: Online Medical Library  
\(^{55}\)
The RAS is outlined in Figure 1. In RAS, juxtaglomerular cells of the kidney release the enzyme renin into circulation in response to low BP. Renin interacts with the zymogen (inactive precursor) Angiotensinogen which is produced in the liver, and converts this to Ang I. Although renin is primarily secreted by the juxtaglomerular cells of the kidney, renin and its inactive form prorenin can also be secreted by other tissues. Next Ang I is further spliced to form Ang II by ACE which is present on many endothelial cells in the kidney and lungs. Ang II is considered the most biologically active component of the RAS, and acts as a potent vasoconstrictor, in addition to signaling the secretion of additional BP elevating hormones such as aldosterone. Ang II exerts its physiological effects by binding to angiotensin II receptor type 1 (AT$_1$R) or angiotensin II receptor type 2 (AT$_2$R) found on endothelial cells of the vasculature, kidney, skeletal muscle, heart, adipocytes, pancreas, brain, and adrenal tissues. When Ang II binds to AT$_1$R, its physiological effects include vasoconstriction, sodium retention in the renal tubules, and aldosterone release from the adrenal gland. Ang II bound to AT$_2$R has opposing effects, and includes vasodilation, anti-inflammatory effects in vascular smooth muscles, and anti-proliferative effects in the myocardium.

Ang 1-7 is produced either when Ang II is cleaved by the enzyme ACE2, which is present in epithelial cells, or when Ang 1-9 is cleaved by ACE. Angiotensin 1-7 (Ang 1-7) has been shown to counteract the effects of Ang II, and therefore acts as a vasodilator. By binding to MAS receptors, Ang 1-7 causes vasodilation via activation of nitric oxide and bradykinin pathways. Because some of Ang 1-7 is a byproduct of Ang II degradation, it is not clear whether the vasodilatory effects are
due to decreasing Ang II, or increasing Ang 1-7 levels; it is likely to be a combination of both, so the ratio of Ang II/Ang 1-7 can serve as a marker of overall activity or balance.\textsuperscript{15}

The hormones of the RAS have systemic effects on blood pressure, as well as proliferative effects on the heart, kidney, and endothelium.\textsuperscript{16} Although the kidney is associated with the release of renin, the enzyme responsible for setting off the rest of the cascade, other tissues have been shown to release renin as well.\textsuperscript{57} Additionally tissues and organs such as skeletal muscle, the pancreas, adipose tissue, the heart, and the brain have been shown to produce many of the RAS components, perhaps even utilizing a complete localized RAS.\textsuperscript{15,56} One study conducted by Pringle et al.\textsuperscript{58} examined the associations of the circulating and intrarenal RAS in healthy adults. The authors found that while urinary renin and angiotensinogen were closely associated with other urinary RAS components, there was no correlation with measures of circulating counterparts.\textsuperscript{58} While urinary angiotensinogen, and other RAS components are good measures of renal RAS activity, serum RAS measurements likely reflects secretion from multiple organ systems such as adipose tissue, skeletal muscle, the heart, etc. and therefore must also be obtained to measure systemic RAS activity.\textsuperscript{15,57}

Little research has examined the relationships between being born PT/LBW and RAS measurements. Three studies were found that examined the relationship between birth measurements and RAS; of the three studies, only two included participants that were either PT or LBW.\textsuperscript{10,11,13}
Forsyth et al. was the first to examine the relationship between birth weight and the concentrations of the RAS enzyme serum ACE. In this prospective study, sixty-eight term-born infants were recruited; all having normal birth weight (3498 g ± 506 g), and normal gestation (281 days ± 8.7 g). Serum ACE was measured at birth, one month, and three months of age. Forsyth et al. found that there was a highly significant negative correlation between birth weight and serum ACE activity at three months of age ($r = -0.52, p < 0.001$), which persisted after adjusting for birth weight z-scores ($r = -0.34, p = 0.03$). The authors concluded that at three months of age, infants with lower birth weight tended to have higher levels of serum ACE activity, and that having higher ACE in early life may program the individual for increased cardiovascular risk.

Franco et al. also looked at RAS measurements and their relationship with birth weight, but did so in eight to thirteen year old adolescents. Sixty-six adolescents were included in the study and were divided into either an appropriate birth weight for gestational age group ($n = 31$, birth weight = 3154 ± 187 g, gestational age = 39 ± 1 week), or a small birth weight for gestational age group ($n = 35$, birth weight = 2346 ± 250 g, gestational age = 39 ± 1). For both groups, blood pressure, catecholamine, and RAS measurements were taken. It was found that both serum Ang II and ACE levels were significantly higher in small for gestational age boys ($p = 0.024$, $p = 0.050$). Additionally, SBP, Ang II, and ACE were found to have a significant negative correlations with birth weight ($r = -0.38, p = 0.001$; $r = -$).
The authors concluded that these results may support a link between birth weight and RAS over activity in childhood.

Lastly, Miyawaki et al. looked at the relationship between birth weight, gestational age, and Ang II levels shortly after birth. This prospective study consisted of forty-six infants who were divided into a normal birth weight (NBW = 2500 – 4000g) group (NBW n = 14, BW = 2958 ± 287g, GA = 39 ± 2 weeks), a LBW group (n = 16, BW = 2014 ± 249 g, GA = 34 ± 2 weeks), and a VLBW group (n = 16, BW = 1001 ± 307 g, GA = 28 ± 3 weeks). Blood samples were collected on day zero, and day seven after birth, and Ang II levels were analyzed. Ang II levels were significantly higher in the VLBW group on day seven compared to the NBW group (p = 0.005) and the LBW group (p = 0.031). Ang II levels on day seven were also significantly correlated with both gestational age (r = -0.4, p = 0.007), and birth weight (r = -0.36, p = 0.016).
FOAD and Nephrogenesis

Nephrogenesis refers to the embryologic origins of the kidney, and the process of development that occurs until organ maturity. In mammals, the kidney develops in three successive phases, the pronephros, mesonephros, and metanephros; each phase increasing in complexity.\(^5^9\) The pronephros is the most immature form of the kidney, and begins development during twenty two days of gestation.\(^5^9\) After 5 weeks of gestation, the mesonephros develops the uretic bud (UB, Figure 2), which grows and branches to form the renal pelvis and collecting ducts of the adult kidney.\(^5^9\) This is the metanephros phase, and after continuous cell differentiation, all of the branching structures of the adult kidney have been formed by 32 to 36 weeks gestation.\(^5^9\) Although uretic branching and nephron units have been formed by this time, these structures are not mature, and continue to mature after birth.\(^5^9,^6^0\)
Prematurity, Low Birth Weight, and the Kidney

The higher BP observed in LBW/PT individuals may be explained in part by FOAD and the effects on the developing kidney, which plays an important role in BP regulation. As mentioned previously, individuals born preterm experience the disruption of crucial developmental periods, and therefore compensate by altering organ system structure and function. In human kidney development the first nephrons begin to form at about 9 weeks of gestation, however the majority of nephrogenesis and kidney development is not complete until later gestation, during the third trimester (28-37 weeks). Because many PT births occur during or before this critical period of kidney development, incomplete kidney formation may therefore play a role in the higher BP reported in populations born PT.

Studies conducted in the PT born baboon model have demonstrated that kidney development continues after PT birth, and that this postnatal development can yield abnormal nephrons. A study conducted by Gubhaju et al. showed that when compared to term born baboons, baboons that were born PT had a significant decrease in glomerular density (number of glomeruli per gram of kidney). Additionally it was found that up to 18% of the glomeruli in the PT kidney were found to be cystic and shrunken in PT baboons (Figure 4). The immature state of development, and poor vascularization is likely to leave the cystic glomeruli non-functional.
Figure 4: Photomicrographs of kidney in Control and PT Baboons
Healthy glomeruli in term born (Left), compared to cystic glomeruli in PT rats (right). The
cystic glomeruli show ruptured capillary and tubule structure, leaving the glomeruli non-
functional. Source: Gubhaju 09

A subsequent review by Gubhaju et al. has found that similar nephrogenesis
may exist in human populations born PT. Gubhaju et al. found that at autopsy,
kidneys from PT born children contain cystic and abnormal glomeruli similar to
those observed in the baboon model. The location of these abnormal glomeruli,
being in the outer cortex of the kidney, imply that they were newly formed following
PT delivery.

One cross sectional study conducted by Rodríguez et al. studied the clinical
and functional renal parameters in 40 children, aged 6-12, who were born
extremely low birth weight (ELBW <1000g). Although ELBW children and controls
had no significant differences in renal length or volume, ELBW children had
significantly higher kidney creatinine concentration, and lower creatinine clearance
compared to term born controls. Furthermore, evidence of tubular phosphate
transport defect were present among ELBW, with ELBW participants having higher
urinary phosphate and calcium excretion. Rodríguez concluded that in this
population of children born ELBW, there was evidence of diminished glomerular
filtration rate, in addition to tubular phosphate transport, and that these results were likely a consequence of impaired postnatal nephrogenesis.

Although the evidence is limited, PT/LBW appears to be associated with impaired kidney structure and function, and may play a role in the higher BP seen in this population. Because the kidney also has an important role in the RAS, a hormonal BP control mechanism, it is necessary to examine if PT/LBW might also have an impaired RAS.
Physical Activity and Cardiorespiratory Fitness

Physical activity (PA) and aerobic fitness both have well documented health benefits, and having higher levels of cardiorespiratory fitness, and/or participating in more PA is associated with a lower risk for cardiometabolic diseases and overall mortality. The beneficial effects of PA are independent of age, sex, and race, as well as the presence of existing cardiovascular disease. Therefore, lifestyle interventions that incorporate PA designed to enhance cardiorespiratory fitness are recommended to prevent the development of cardiometabolic disease and hypertension. It has been suggested that individuals born PT/LBW are at increased risk for developing hypertension, however having lower levels of cardiorespiratory fitness, and/or PA participating may have contribute to or even mediate this risk in this population as well.

Aerobic Fitness and Physical Activity in PT/VLBW

Several studies have reported on the relationships between prematurity, birth weight, aerobic fitness, and physical activity. Six studies examined this relationship in study populations that were PT/VLBW, six studies included participants who were EPT/ELBW, and one study stratified participants into PT, VLBW, and ELBW groups. Although a majority of studies report lower exercise capacity across a spectrum of BW and GA, other studies report conflicting results.
Three studies examined aerobic capacity and/or PA in PT/VLBW populations \cite{23,25,63}. In one study, Vrijlandt et al. examined the relationship between being born PT/VLBW and exercise capacity in ninety young adults (18-22 yr) \cite{25}. Although there was no difference in VO$_2$ max between the PT/VLBW group (n=42, age=19-20yr) and the control group (n=48, age=18-22), PT/VLBW participants had significantly lower anaerobic threshold, as well as lower exercising work efficiency compared to their term born peers, as determined by a maximal cycle ergometer test \cite{25}. Vrijlandt and colleagues found only minor respiratory impairment in the PT/VLBW group, therefore lung function was not considered to be a limiting factor of exercise capacity \cite{25}.

Three other studies also examined aerobic fitness in PT/VLBW individuals and reported conflicting results \cite{23,25,63}. Baraldi et al. assessed the cardiorespiratory and metabolic response to exercise in fifteen PT/VLBW 7-12 year olds compared to healthy peers (n=26), and also compared exercise performance in individuals who were appropriate for gestational age (AGA= birth weight between 10$^{th}$ and 90$^{th}$ percentile) (n=9) and small for gestational age (SGA = birth weight less than 10$^{th}$ percentile) (n=6) \cite{63}. The authors found that PT/VLBW children did not have different VO$_2$ peak values, determined by an incremental treadmill test, compared with controls; however children who were SGA had significantly higher energy cost of running (p < 0.025) \cite{63}. Baraldi et al. concluded that these findings might be due to a delay in muscle coordination mechanisms observed in SGA children \cite{63}. Also Barker and colleagues also reported no differences between VO2max in PT/VLBW and term born controls, assessed by a maximal cycle ergometer test \cite{23}. 
Barker found that 26 PT/VLBW adolescents did not have significantly different VO2 max values compared to term born controls. Additionally, Barker and colleagues reported significantly lower work rates in PT/VLBW individuals, and concluded that these abnormalities seen at peak exercise were due to ventilatory limitations such as lower ventilatory threshold and oxygen desaturation.

Lastly, Tsopanoglou et al. reported on the functional exercise capacity of thirty-seven PT/VLBW children (6-9 yr) compared to term born controls, as assessed by a six-minute walk test, and a 10 meter shuttle walk test. The authors found that PT/VLBW children walked shorter distances for the six-minute walk test (p = 0.010) compared to term controls, and therefore had limited functional capacity. This was especially true for PT/VLBW individuals who had bronchopulmonary dysplasia -chronic lung damage caused by mechanical ventilation- and oxygen dependence at 28 days after birth.

Two studies reported on the exercise and PA habits among PT/VLBW individuals. Both Kajanti et al. and Kaseva et al. assessed PA participation using standardized questionnaires, and reported that their study populations of formerly PT/VLBW young adults (21-29 yrs) reported less exercise participation compared to healthy peers. Both authors conclude that unimpaired PT/VLBW individuals participate in less leisure time PA compared to healthy peers, and that exercise promotion could be an important preventative measure in this population at higher risk for CVD.
Aerobic Fitness and Physical Activity in EPT/ELBW

Six studies reported on either aerobic fitness or exercise capacity in formerly EPT/ELBW individuals, where one study also examined the exercise habits in this population.

In one study, Smith et al. examined the long term respiratory and fitness outcomes in children who were born EPT/ELBW. One hundred twenty-six ET/ELBW children participated in the study and were compared to term born controls; mean (SD) gestational age for EPT/ELBW was 26.9 (1.7) weeks, and birth weight was 862 (161) g; gestational age for controls was 39.4 (1.2) weeks, and birth weight was 3400 (513) g. Measurements were taken when the participants were at a mean age of 10 years, and consisted of spirometry, lung volumes, and gas exchange to measure lung function; and a 6-minute walk, and shuttle run to assess cardiorespiratory fitness. Smith and colleagues found that the EPT/ELBW group had significantly lower measures of cardiorespiratory fitness, despite only mild impairments in lung function compared to the control group. Because lung function was not significantly impaired in the EPT/ELBW group, it was not likely that lung function was a limiting factor of exercise capacity.

Four additional studies examining aerobic fitness and exercise capacity in EPT/ELBW individuals reported similar results. Three of these studies used maximal graded treadmill testing, or cycle ergometry to assess aerobic fitness, and found that EPT/VLBW individuals had significantly lower VO2 max values compared to healthy controls. In the fourth study, Rogers et al. assessed fitness using the Modified Canadian Aerobic Fitness Test, and reported lower
aerobic fitness capacity in EPT/ELBW vs. controls. Lower participation in PA, impaired motor coordination, and parents’ hesitation to promote exercise were all listed as potential factors influencing this relationship. 

Lastly, Clemm et al. assessed aerobic exercise capacity and performance in the EPT/ELBW population, and reported conflicting results. Aerobic fitness was measured using maximal graded treadmill testing, and was conducted on seventy-five EPT/ELBW, and seventy-five controls; both groups had a mean age of 17.6 years. Clemm reported that there were no significant differences in aerobic fitness between EPT/ELBW individuals, and term controls.

**Summary**

Among the four studies examining aerobic fitness and exercise capacity in PT/VLBW individuals, three studies reported lower exercise capacity, one study reported a higher energy cost of running, and one study reported lower aerobic threshold (a marker of aerobic fitness), compared to healthy term controls. Only three of the four studies directly measured aerobic fitness (VO$_2$ max) via graded exercise tests, with all reporting no significant differences in VO$_2$ max between PT/VLBW and term controls. Additionally, two studies reported on the leisure time physical activity habits of PT/VLBW individuals, and both reported significantly lower participation in this population.

Six studies investigated aerobic fitness and capacity in EPT/ELBW participants, where two studies reported lower exercise capacity, three studies
reported lower VO₂ max⁶⁶,⁶⁷,⁶⁹, and one study reported no difference in VO₂ max, compared to healthy term controls²⁹.

The evidence examining the relationship between birth weight and gestational age, aerobic fitness, and physical activity consists of conflicting findings, but seems to suggest increased impairment for those with lower birth weights and/or gestational ages. Inconsistent measurements, and inappropriate exercise protocols may be responsible for these conflicting results.

Physical activity interventions have proven to be effective in lowering cardiovascular disease risk factors, such as high BP in healthy populations²⁰. Because PT/VLBW individuals have been shown to have higher BP than their term born peers, and because this population may have lower cardiorespiratory fitness, it may be especially appropriate to implement PA interventions for this at risk population⁶,²⁰,²⁴.

Effects of Exercise on the Renin Angiotensin System

It is well established that exercise and PA lowers cardiovascular disease risk, by reducing various risk factors such as BP²⁰. A recent study has shown that exercise may reduce circulating inflammatory biomarkers⁷¹, perhaps contributing to lowering BP; however there has only been little research examining the relationships that PA may have with the BP elevating hormones of the RAS. Because of the powerful effects that the RAS has on BP, it is important to determine if
exercise and PA help to lower the vasoconstrictive components of the RAS in this at risk PT/LBW population.

There have been some studies examining the effects of exercise on RAS in animal models. Agarwal and colleagues have investigated this relationship using a spontaneously hypertensive rat (SHR) model. For this study, seven week old, healthy Wistar-Kyoto rats (WK), and SHR rats were randomized into either a sedentary group, or an exercise group. Rats in the exercise group underwent an intervention consisting of five days a week of moderate running on a motorized treadmill, for one hour per session. The investigators found that SHR rats in the exercise group had significantly lower SBP, DBP, and MAP when compared with SHR rats that were sedentary. Furthermore, while SHR rats demonstrated an imbalance of RAS expression in cardio-regulatory brain regions at baseline, after exercise SHR demonstrated lower expression of ACE, and higher ACE2 and Mas in these brain regions compared to sedentary SHR rats. Exercising SHR rats also had significantly lower serum Ang II compared to sedentary SHR rats. These effects were only seen in the SHR rat model, and no significant changes in RAS were noted in the healthy WK rats between the pre and post exercise periods. These results suggest that chronic exercise may up regulate the vasoprotective components of the RAS, while down regulating the vasoconstrictors.

There has been some research examining the relationship between exercise and RAS in humans. Goessler et al. included eleven studies on this topic in a systematic review and meta-analysis. The included randomized control trials consisted of a total of 375 healthy adults, with a median age of 52.5 years, and
ranging from 22-68 years. After analysis, the authors conclude that exercise training resulted in significantly lower plasma renin activity (p=.049), but did not significantly affect levels of Ang II or serum aldosterone in healthy adults. It is important to note that only three of the eleven studies in Goessler’s analysis included data on Ang II, one of the studies only included women, another study focused only on resistance training and the third study included both resistance and aerobic training. Plasma renin activity has been shown to be lower among athlete populations, and higher in populations of hypertensive individuals. While the meta-regression analysis conducted by Goessler did not show a relationship between BP and RAS measurements, the authors concluded attenuated BP by exercise is likely due to multiple factors, including the hormones of the RAS. Additionally, the relationship between BP and RAS may be different in different populations, and could also be dependent on exercise intensity and dose. Lastly, although no significant decrease in Ang II or aldosterone was found after exercise training, only three small studies examined these parameters; therefore a lack of statistical power to detect changes could have been responsible for these observations.

To date, no research has studied the effects of exercise on the RAS in PT/LBW individuals. Because this population has been reported to have increased RAS markers, lower measures of cardiorespiratory fitness and PA, and higher CVD risk, it is important to continue to study the role of fitness and PA in this population.
Summary

There is evidence demonstrating that individuals who are born PT with lower birth weight have increased levels of ACE and Ang II, components of the RAS leading to vasoconstriction. Additionally, there is evidence suggesting that PT/VLBW individuals have higher BP and increased cardiometabolic risk, in addition to lower measures of PA and cardiorespiratory fitness. Some evidence suggests that exercise training lowers levels of Ang II and ACE in animal studies; while human randomized trials have shown that exercise lowers plasma renin activity. There are no studies examining the potential role that physical activity and/or cardiorespiratory fitness may play on the association between PT/VLBW birth and RAS.
Purpose

The purpose of this thesis was to examine the levels of PA and aerobic fitness and markers of RAS in preterm VLBW adolescents compared with term-born normal birth weight (NBW) peers, and to determine the influence of PA and fitness on RAS. It was hypothesized that PT/VLBW adolescents would have lower levels of fitness and PA participation, and higher plasma Ang II/Ang1-7 ratios compared to term NBW peers, and that lower fitness/PA participation may partially mediate higher ratios. Additionally, it was hypothesized that there would be no differences in urinary RAS measurements between PT/VLBW and NBW peers.
METHODS

Participants

Participants in this study consisted of fourteen-year-old adolescents who volunteered for the Prenatal Events – Postnatal Consequences (PEPC) study, a study examining antenatal corticosteroid exposure on BP and systems associated with its regulation. Two study groups were included, a VLBW group, and a NBW term-born group serving as the healthy controls.

VLBW participants were located using a computer database. Once the participant was located, a postcard was mailed asking the parent/guardian whether or not their child would be interested in participating in the proposed study. Telephone calls were made for any participant who did not respond to the initial postcard. Two hundred VLBW participants were proposed to be recruited. Eligibility criteria for VLBW participants was 1) birth occurred between 01/01/1992 to 06/30/1996; 2) birth weight < 1501 grams; 2) hospital of birth was Forsyth Medical Center; 3) singleton birth; 4) and follow up visit at one year adjusted age.

Term-born NBW controls were recruited in accordance with the race and gender distribution of the VLBW participants. Advertisements made via local newspapers, magazines, and internal postings outlined the general inclusion criteria; responders were then screened for eligibility. Eligibility criteria for NBW term controls included 1) birth occurred between 06/01/1994 to 6/30/1996, 2) were in their fifteenth year of life at all three visits; 3) hospital of birth was Forsyth
Medical Center; 4) gestational age was ≥ thirty-seven weeks; 5) birth weight was ≥2500 grams; 6) and singleton birth.

Adolescents from either group were excluded from participation if they had any major congenital abnormality, or if they had any contraindication to exercise testing on a cycle ergometer. Written informed consent was obtained from a parent or guardian, and written assent was obtained from each adolescent according to Wake Forest University Baptist Medical Center (WFUBMC) Institutional Review Board (IRB) guidelines. Each adolescent was compensated $50, $75, and $100 ($225 total) for the first, second, and third visits, respectively. Parents were compensated $25 in travel expenses for each visit.

Procedures and Measurements

Once enrolled, participants along with their parent or legal guardian reported to the General Clinical Research Center (GCRC) at WFUBMC for each visit. Written informed consent form the parent and assent from the adolescent were obtained during the first visit. Measurements performed at each visit are summarized in Table I. Visits were typically scheduled at least four weeks apart; however some flexibility according to participants' schedule was permitted. Detailed methods and procedures are limited to those relevant to this thesis project, which are shown in bold.
**Table I: PEPC Measurements**

<table>
<thead>
<tr>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Written informed consent &amp; written assent</td>
<td>Home urine sample</td>
<td>Urine sample (same as V1 + Ang II, Ang 1-7, ACE, ACE 2, and pregnancy test for females)</td>
</tr>
<tr>
<td>Height, weight, MUAC measured in triplicate</td>
<td>Height, weight, MUAC measured in triplicate</td>
<td>Height, Weight &amp; MUAC measured in triplicate</td>
</tr>
<tr>
<td>Stage of sexual maturation assessment</td>
<td>Stage of sexual maturation assessment</td>
<td>Stage of sexual maturation assessment</td>
</tr>
<tr>
<td>Resting BP</td>
<td>Resting BP</td>
<td>Resting BP &amp; ambulatory BP set up (optional)</td>
</tr>
<tr>
<td>Urine sample: Na⁺, K⁺, microalbumin, creatinine</td>
<td>Maximal progressive exercise test for aerobic fitness VO₂peak &amp; PWC</td>
<td>Blood sample: serum measurements of renin, aldosterone, blood electrolytes, creatinine, angiotensin peptides, and ACE</td>
</tr>
<tr>
<td>Salivary cortisol pre &amp; post cold pressor</td>
<td>Salivary cortisol pre &amp; post GXT</td>
<td>Skinfold measurements</td>
</tr>
<tr>
<td>Cold pressor test</td>
<td>Pulmonary function tests</td>
<td>Dual Energy X-ray Absorptiometry scan: % body fat, bone density, &amp; lean body mass</td>
</tr>
<tr>
<td><strong>Modifiable Activity Questionnaire (MAQ)</strong></td>
<td></td>
<td>BOTMP</td>
</tr>
<tr>
<td>Resting ECG (HRV)</td>
<td>Food Record Instructions</td>
<td>Collect food record</td>
</tr>
</tbody>
</table>

**Anthropometry**

During each visit, height, weight, and mid upper arm circumference (MUAC) were measured in triplicate by a trained GCRC nurse. Height was measured to the nearest tenth of a centimeter without shoes on using a wall-mounted stadiometer. Weight was measured to the nearest tenth of a kilogram using a digital platform scale, and was collected while participants wore light clothing and no shoes. BMI
was determined by dividing the average weight (kg) by the average height squared (m²). All measurements were recorded on the appropriate Data Collection/Protocol Instruction Sheet corresponding to the visit day. BMI percentile was determined from CDC 2000 reference data and ≥ 85th percentile was considered overweight or obese.75

Aerobic Fitness

Each participant underwent a graded exercise test to exhaustion on a cycle ergometer following the Godfrey Protocol.76 Aerobic fitness was determined from the collection of expired gases using the VMAX Encore Metabolic Cart. Peak oxygen uptake (VO₂ peak) was taken from the last thirty seconds of exercise, and was expressed per kg of body mass. Throughout testing a twelve-lead ECG was monitored continuously during exercise, and for five minutes post exercise. Testing was terminated in the presence of any ECG or other physiologic abnormalities in accordance with the guidelines established by the American College of Sports Medicine.77 Participants were verbally encouraged to give a maximal effort, and testing was terminated when the participant could no longer maintain a pedaling frequency of forty rpm. VO₂peak % of predicted was determined from age and sex-specific reference values.78
Physical Activity

Physical activity over the past year was assessed using Kriska’s Modifiable Activity Questionnaire (MAQ)\textsuperscript{79}. The MAQ was administered directly to participants, with parents present if assistance was needed. Participants were read a list of activities, and were asked to identify any activity that they participated in at least five times in the past year. For each activity, they were asked to report which months during the past year that they participated in that activity, as well as the average number of times per month or week, and average amount of time per session. Habitual activity was determined from the average of total hours of activity per week (TOT-hrs) for the past year and the past two months. An estimate of relative intensity, expressed as MET hours per week, was calculated for each activity by multiplying the number of hours per week spent in that activity by the metabolic cost of that activity (obtained from existing tables) to obtain MET-hours per wk (MET-hrs).\textsuperscript{80} The average number of hours spent in activities with estimated MET values greater than six was classified as vigorous activity (VIG-hrs). Part of the questionnaire was focused specifically on PA that took place in the two months preceding the study visit; this was to provide a measure of PA closer to the date of fitness testing and RAS measurements.

Renin Angiotensin System

Measures of RAS were assessed via both urine samples and blood samples. Urine was collected on visit three using a clean catch procedure in 1 N HCL, to obtain measurements of Ang II, Ang 1-7, ACE, ACE 2, and creatinine. Urine was
extracted on Sep-Pak c18 cartridges (200mg Waters Corp. Milford, MA). Sep Pak cartridges were initially activated with 5 ml 90% ethanol (90%), 4% acetic acid, 3 ml methanol, and 5 ml 4% acetic acid; peptides were eluted with 10 ml 90% ethanol/4% acetic acid. Urinary measurements were expressed per gram of creatinine.

Blood samples were collected into chilled Vacutainer collection tubes containing a mixture of peptidase inhibitors. After being chilled for twenty minutes on ice, the samples were centrifuged at 3000 rpm for twenty minutes at 4 degrees Celsius, and plasma drawn without disturbing the packed cells.

Blood and urine samples were analyzed for angiotensin peptides, renin, and aldosterone content via radioimmunoassay (RIA) using methods were described previously by Chappell and colleagues. Ang I was measured using a modified New England Nuclear RIA kit, Ang II was measured using a Nichols Institute RIA kit, and Ang 1-7 was measured as described by Chappell et al. The ratio of AngII/Ang 1-7 was calculated for both urinary and blood measures to provide a measure of dominant RAS activity.

**Birth Weight and Neonatal Information**

For VLBW adolescents, medical records available through Forsyth Medical Center were reviewed to obtain neonatal characteristics including birth weight, and gestational age. Birth weight z-score was determined from gestational age and sex-specific reference data. For term-born participants, information regarding birth
weight and gestation age was obtained from the delivery room log at Forsyth Medical Center.

**Statistical Analysis**

Statistical analyses were performed using SPSS for Windows (version 22.0). Descriptive analyses, including measures of central tendency and dispersion, were conducted on variables of interest to determine if parametric analyses were appropriate. Data that were not normally distributed were transformed using natural log or square root to improve distributional characteristics. Independent sample t-tests were performed to examine between-group differences. Pearson correlational analysis was used to assess the relationship among variables of interest. Multiple regression analysis was used to determine $\beta$ coefficients for the associations among variables. Meditational analysis was used to examine the potential mediating effect of fitness on the association between PT birth and RAS markers.
RESULTS

Participants

From a cohort of 479 PT/VLBW who met the inclusion criteria, a total of 193 were enrolled in the PEPC study. Of the 193 PT/VLBW participants, seven participants were found to be ineligible, and were excluded after enrollment. An additional 16 PT/VLBW participants were excluded from analyses due to unreliable physical activity data. Fifty-two term-born NBW controls were initially enrolled, however two did not complete all three study visits, and one was unable to complete exercise testing due to an ACL injury. The final sample included in analysis was comprised of 170 PT/VLBW, and 48 term participants who had provided urine samples, and had valid PA data.

Characteristics for 170 VLBW and 48 term participants are presented in Table 2. All participants were 14 years of age at the time of data collection. Of the 170 PT/VLBW adolescents, 44% were male, and 58% were non-black. Of the 48 term-born participants, 44% were male, and 62% were non-black. Compared to term-born peers, PT/VLBW had significantly lower birth weight, with 44% being classified as ELBW (<1000kg). Additionally birth weight z-scores, gestational age, height z-score, and weight z-score were also significantly lower in PT/VLBW; however BMI z-scores did not differ between groups. Based on the CDC 2000 reference data, 34% of PT/VLBW, and 33% of term participants had BMI ≥ 85th percentile, classifying these participants as overweight or obese.
<table>
<thead>
<tr>
<th></th>
<th>PT/VLBW</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>170</td>
<td>48</td>
</tr>
<tr>
<td>Gender % male n(%)</td>
<td>74(44)</td>
<td>21(44)</td>
</tr>
<tr>
<td>Race n(%) non black</td>
<td>99(58)</td>
<td>30(62)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1062 ± 267 *</td>
<td>3463 ± 484</td>
</tr>
<tr>
<td>Birth weight z-score</td>
<td>-0.29 ± 0.84 *</td>
<td>0.004 ± 1.06</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>27.9 ± 2.6 *</td>
<td>39.6 ± 1.1</td>
</tr>
<tr>
<td>Age at follow up (years)</td>
<td>14.5 ± 0.3</td>
<td>14.6 ± 0.3</td>
</tr>
<tr>
<td>Height z-score</td>
<td>-0.39 ± 1.11 *</td>
<td>0.54 ± 0.93</td>
</tr>
<tr>
<td>Weight z-score</td>
<td>0.32 ± 1.30 *</td>
<td>0.86 ± 0.82</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>0.46 ± 1.30</td>
<td>0.69 ± 0.80</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>62 ± 170</td>
<td>70 ± 22</td>
</tr>
</tbody>
</table>

* p < 0.05 for Ln transformed data
Aerobic Fitness and Physical Activity

Aerobic fitness and physical activity characteristics are presented in Table 3. Fitness data of 13 PT/VLBW participants were excluded from analysis due to cardiac abnormalities during testing (n = 4), technical error (n = 5), and submaximal effort (n = 4), leaving 157 participants for analysis. Forty-nine term controls were included in VO₂ analysis. Compared to term born peers, PT/VLBW participants had significantly lower aerobic fitness when expressed in ml/kg/min as well as percent of predicted. Furthermore, 60% of PT/VLBW had VO₂peak values < 80% of predicted (based on sex and age) compared to only 33% of term controls.

Of the 170 PT/VLBW and 48 term participants who had reliable PA data, PT/VLBW subjects participated in significantly fewer VIG-hrs than term controls, but TOT-hrs did not differ between groups. A total of 58% of PT/VLBW and 63% of term participants met the national recommendation of 7 hours of PA per week, however only 32% of PT/VLBW and 54% of term met the national recommendation for vigorous PA (2.25 hrs/wk).

Participation in PA over the past two months was also examined to provide a measure of PA closer to the measurement of fitness and RAS. These results were similar in that PT/VLBW participants reported significantly lower participation in VIG-hrs in the past two months, but Tot and MET hrs did not differ between groups.
Table 3: Aerobic Fitness\textsuperscript{a} and Physical Activity\textsuperscript{b} (expressed as mean ± SD)

<table>
<thead>
<tr>
<th>Measure</th>
<th>PT/VLBW</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO\textsubscript{2} peak ml/kg/min</td>
<td>38.0 ± 10.1 *</td>
<td>41.8 ± 10.8</td>
</tr>
<tr>
<td>VO\textsubscript{2} peak % predicted value</td>
<td>82.0 ± 19.3 *</td>
<td>89.8 ± 19.4</td>
</tr>
<tr>
<td>Tot PA past year (hrs/wk)</td>
<td>10.6 ± 8.7</td>
<td>9.7 ± 6.0</td>
</tr>
<tr>
<td>Vig PA past year (hrs/wk)</td>
<td>2.7 ± 4.2 *</td>
<td>3.9 ± 4.2</td>
</tr>
<tr>
<td>MET hrs past year (hrs/wk)</td>
<td>55.0 ± 47.7</td>
<td>56.6 ± 38.0</td>
</tr>
<tr>
<td>Tot PA past 2 months (hrs/wk)</td>
<td>11.3 ± 11.7</td>
<td>12.0 ± 14.5</td>
</tr>
<tr>
<td>Vig PA past 2 months (hrs/wk)</td>
<td>2.7 ± 5.4 *</td>
<td>4.1 ± 5.5</td>
</tr>
<tr>
<td>Met hrs past 2 months (hrs/wk)</td>
<td>58.1 ± 62.4</td>
<td>64.1 ± 57.3</td>
</tr>
</tbody>
</table>

\textsuperscript{a}n=157 PT/VLBW, 48 term for fitness data; \textsuperscript{b}n = 170 PT/VLBW and 48 Term for PA data. * = p < 0.05 for Ln transformed data.

Measures of the Renin Angiotensin System

Measures of urinary and serum RAS are reported in Table 4. Urine was collected on 170 PT/VLBW participants, and 48 Term participants. Fewer participants volunteered to provide a blood sample; therefore sample sizes were lower for serum and plasma RAS measurements, and are described in Table 4. Compared to term controls, urinary Ang 1-7 was found to be significantly higher in PT/VLBW participants, while no differences between groups were found for urinary Ang II or the ratio of urinary Ang II:Ang 1-7. Measures of serum renin and aldosterone did not differ between groups. Plasma Ang II was also similar between
groups, however PT/VLBW participants had significantly lower plasma Ang 1-7 values, and a significantly higher plasma AngII:Ang 1-7 ratio.

### Table 4: Renin Angiotensin System Components (expressed as mean ± SD)

<table>
<thead>
<tr>
<th>Component</th>
<th>PT/VLBW</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary Ang II/Creatinine&lt;sup&gt;a&lt;/sup&gt; (pmol/g)</td>
<td>17.90 ± 37.65</td>
<td>14.22 ± 33.99</td>
</tr>
<tr>
<td>Urinary Ang 1-7/Creatinine&lt;sup&gt;b&lt;/sup&gt; (pmol/g)</td>
<td>76.42 ± 46.94 *</td>
<td>65.54 ± 48.06</td>
</tr>
<tr>
<td>Urinary Ang II:Ang 1-7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.23 ± 0.31</td>
<td>0.31 ± 0.71</td>
</tr>
<tr>
<td>Plasma Renin, (nmol Ang I/L/hr)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.46 ± 1.56</td>
<td>2.51 ± 1.85</td>
</tr>
<tr>
<td>Plasma Aldosterone, (pmol/L)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.74 ± 7.60</td>
<td>9.70 ± 6.64</td>
</tr>
<tr>
<td>Plasma Ang II, (pmol/L)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>25.43 ± 12.72</td>
<td>29.60 ± 14.58</td>
</tr>
<tr>
<td>Plasma Ang 1-7, (pmol/L)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>8.52 ± 9.06 *</td>
<td>13.30 ± 7.99</td>
</tr>
<tr>
<td>Plasma Ang II:Ang 1-7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.81 ± 8.50 *</td>
<td>3.44 ± 3.38</td>
</tr>
</tbody>
</table>

n for PT/V:BW:Term:<sup>a</sup> = 170:48; <sup>b</sup> = 170:47; <sup>c</sup> = 118:43; <sup>d</sup> = 119:43; <sup>e</sup> = 117:42; <sup>f</sup> = 118:42  * = p < 0.05 for Ln transformed data.

### Correlational Analysis

Pearson Correlation analysis was used to examine associations between aerobic fitness and PA and RAS measurements separately for PT/VLBW and term groups. Table 5 shows correlation coefficients for PA and fitness with urinary RAS, however no significant correlations existed for PT/VLBW or term groups.
Correlation coefficients with circulating RAS measures are shown in Table 6. Measures of circulating RAS did not correlate with fitness or PA within Term control participants. However, in the PT/VLBW group, plasma Ang II and the Ang II: Ang 1-7 ratio were significantly inversely correlated with VO$_2$ peak and VO$_2$ % of predicted. Serum aldosterone was found to have a significant correlation with both Vig hrs and MET-hrs.
Table 5: Pearson Correlation Coefficients between Aerobic Fitness, Physical Activity, and Urinary RAS.*

<table>
<thead>
<tr>
<th></th>
<th>Ang II (pmol/g creatinine)</th>
<th>Ang 1-7 (pmol/g creatinine)</th>
<th>Ang II:Ang 1-7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PT/VLBW</td>
<td>Term</td>
<td>PT/VLBW</td>
</tr>
<tr>
<td>VO2peak (ml/kg/min)</td>
<td>.116</td>
<td>-.044</td>
<td>.073</td>
</tr>
<tr>
<td>VO2 (% predicted)</td>
<td>.109</td>
<td>-.004</td>
<td>.076</td>
</tr>
<tr>
<td>Tot PA (hrs/week)</td>
<td>-.031</td>
<td>-.027</td>
<td>.076</td>
</tr>
<tr>
<td>Vig PA (hrs/week)</td>
<td>.063</td>
<td>.052</td>
<td>.023</td>
</tr>
<tr>
<td>Met hrs PA (hrs/week)</td>
<td>-.015</td>
<td>-.029</td>
<td>.087</td>
</tr>
</tbody>
</table>

Data shown are Pearson Correlation r values. RAS, Fitness, and physical activity was log transformed for analysis. Tot PA = past year average of total hours of physical activity per week; Vig PA = vigorous hours physical activity; Met PA = MET hours of physical activity. * All p>0.05
<table>
<thead>
<tr>
<th></th>
<th>Renin (nmol Ang I/L/hr)</th>
<th>Aldosterone (pmol/L)</th>
<th>Ang II (pmol/L)</th>
<th>Ang 1-7 (pmol/L)</th>
<th>Ang II:Ang 1-7 (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PT/VLBW</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Term</strong></td>
<td>.034</td>
<td>.027</td>
<td>-.0124</td>
<td>.030</td>
<td>-.257 *</td>
</tr>
<tr>
<td><strong>VO₂peak (ml/kg/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PT/VLBW</strong></td>
<td>.052</td>
<td>.034</td>
<td>-.099</td>
<td>.034</td>
<td>-.207 *</td>
</tr>
<tr>
<td><strong>Term</strong></td>
<td>-.056</td>
<td>-.138</td>
<td>-.141</td>
<td>.014</td>
<td>-.032</td>
</tr>
<tr>
<td><strong>VO₂peak (% predicted)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PT/VLBW</strong></td>
<td>.067</td>
<td>.070</td>
<td>-.223 *</td>
<td>.135</td>
<td>-.123</td>
</tr>
<tr>
<td><strong>Term</strong></td>
<td>-.045</td>
<td>-.168</td>
<td>-.187 *</td>
<td>.022</td>
<td>-.049</td>
</tr>
<tr>
<td><strong>Tot PA (hrs/wk)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PT/VLBW</strong></td>
<td>.052</td>
<td>.034</td>
<td>-.099</td>
<td>.034</td>
<td>-.207 *</td>
</tr>
<tr>
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<td>-.138</td>
<td>-.141</td>
<td>.014</td>
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<td><strong>Vig PA (hrs/wk)</strong></td>
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<td>.070</td>
<td>-.223 *</td>
<td>.135</td>
<td>-.123</td>
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<td>-.168</td>
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<tr>
<td><strong>Met hrs PA (hrs/wk)</strong></td>
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Data shown are Pearson Correlation r values. RAS, Fitness, and physical activity was log transformed for analysis; Met Hrs was square root transformed. Tot PA = past year average of total hours of physical activity per week; Vig PA = vigorous hours physical activity; Met PA = MET hours of physical activity. *p<0.05
Mediational Analysis

Regression analysis was used to determine the influence of aerobic fitness on the relationship between PT/VLBW and RAS. Figure 7 outlines the results of this analysis. PT/VLBW was significantly associated with plasma Ang II:Ang 1-7 ratio as shown with C. Addition of fitness (V\text{O}_2\text{ peak in ml/kg/min}) to the regression model attenuated the PT – AngII:Ang 1-7 ratio relationship by 11%, as indicated by the decrease in beta from .256 to .227 (as shown with C'). Inclusion of race and sex did not change the results appreciably (β = .223). These results indicate partial mediation of this relationship by fitness.

![Figure 7: Mediational Analysis](image)

Beta coefficients from regression analysis are reported. Beta coefficient for the association of PT/VLBW with Ang II/Ang I decreased by 11% (.256 → .227) when adding V\text{O}_2\text{ peak to the regression equation, indicating partial mediating effect.}
DISCUSSION

To our knowledge, this study is the first to compare aerobic fitness, PA, and RAS in a cohort of adolescents born PT/VLBW with their term born peers. Consistent with our hypothesis, we found that PT/VLBW participants had significantly lower fitness and a higher ratio of plasma Ang II:Ang 1-7 than their term born peers. The higher ratio of plasma Ang II:Ang 1-7 observed in our sample was primarily driven by significantly lower levels of plasma Ang 1-7. Our results also suggest that some of the PT/VLBW-Term differences in Ang II:Ang 1-7 may be mediated by aerobic fitness. No differences between groups were observed for the other components of plasma RAS such as renin or aldosterone, or components of the urinary RAS.

Correlational analysis revealed that aerobic fitness was significantly correlated with plasma Ang II, as well as the ratio of plasma Ang II:Ang 1-7 within PT/VLBW subjects. To our knowledge there is no previous research examining the association between aerobic fitness and plasma AngII:Ang 1-7 ratio; however some studies have reported on the effects of exercise training on some components of the RAS. In a meta-analysis of eleven randomized control trials, Goessler et al.\textsuperscript{26} reported that exercise training resulted in a significant reduction in plasma renin activity, but no reduction in plasma Ang II or aldosterone. It is important to note that only three of the eleven studies in Goessler’s analysis included data on Ang II, one of the studies only included women,\textsuperscript{72} another study focused only on resistance
training,73 and the third study included both resistance and aerobic training.74 Furthermore, none of the studies focusing on Ang II included vigorous exercise, which is most closely associated with aerobic fitness.21,26 An additional meta-analysis reported significantly lower levels of renin post training in 10 trials of healthy adults.85 In our study, neither fitness nor PA was correlated with renin activity, however both MET-hrs and VIG-hrs of PA were correlated with plasma aldosterone in the PT/VLBW group only. This finding is inconsistent with the review by Goessler et al. who reported no association in the seven studies examining this relationship in healthy adults.26 Because no significant differences in aldosterone were found between groups, alternate pathways may exist between PA and aldosterone for the PT/VLBW group. It's possible the inconsistent results across studies may reflect differences in age and gender of study samples, as well as differences in the type and length of exercise training.26

In our PT/VLBW group, Ang 1-7 was found to be lower, and responsible for the higher Ang II:Ang 1-7 ratio compared to term. To date, one study has examined PA and Ang 1-7 using an animal model. Argawal et al. 27 suggests that exercise training may reduce the expression of circulating ACE and Ang II, while up regulating the Ang 1-7 receptor expression. In contrast, we did not find a correlation between fitness or PA and Ang 1-7 in either of our study groups.

It is also possible that fitness may indirectly alter the RAS via its effect on body adipose tissue and skeletal muscle. Adipose tissue has previously been described as an endocrine organ, in the sense that adipose tissue is capable of releasing its own RAS components into the blood stream as well as inflammatory
markers. Higher levels of aerobic fitness are generally correlated with higher percentages of lean tissue, and less adipose tissue. Circulating plasma RAS components likely reflect RAS such as Ang II, Ang 1-7, renin, etc, which are secreted by many organs including adipose tissue, the kidneys, the liver, skeletal muscle, and the endothelium. Therefore, it’s possible that alterations in circulating RAS may be due in part to fitness-induced changes in body composition.

In contrast to circulating RAS, we found no relationship between fitness and urinary RAS. Previous reports suggest that although the kidney contributes renin to the circulating RAS pathway, levels of circulating RAS are not correlated to urinary RAS, and urinary RAS is more reflective of the intrarenal RAS system. Lack of association of PA with RAS may also be partially explained by the subjective method of measuring habitual PA using a self-report questionnaire which may have been affected by recall error.

The lower fitness in our PT/VLBW cohort is contrary to three previous studies which reported no difference in fitness between VLBW and term NBW individuals. However, our results are consistent with studies that focused on persons born ELBW, which comprised 44% of our sample. Our findings showed that 50% of PT/VLBW had aerobic fitness lower than 80% of their predicted value; inversely only 33% of term controls had values lower than 80% predicted. These findings may be partially explained by the altered composition of skeletal muscle fiber types, and reduced expression of oxidative phosphorylation genes reported in individuals born with LBW. Contrary to two studies examining exercise and PA in the formerly PT/VLBW population, our results indicate no differences in total
hours of PA between PT/VLBW and term control groups. However we did find significantly less participation in vigorous PA in the PT/VLBW group, which is consistent with previous findings. While 58% of our PT/VLBW subjects met the national recommendation of seven hours per week of PA, 68% of this group participated in less than 2.25 hours of vigorous PA per week. Recent studies have suggested that participation in PA has been on the decline among adolescent populations. Furthermore, a study examining parental awareness of national PA guidelines reports that only 35% of parents are aware of PA guidelines for adolescents. Because the PT/VLBW population is already at an increased risk for developing chronic disease, lower participation in vigorous PA is a cause for concern.

The lower participation in Vig PA may be attributable to several factors. Studies examining fitness and exercise in the PT/VLBW population have found decreased neuromotor coordination and inefficient running economy within this group, which may negatively impact participation in vigorous PA. Performing poorly in vigorous PA, as a result of poor coordination, may also affect their physical activity self-efficacy, making them even less likely to participate. Furthermore, overprotective parents might further discourage their children from participating in PA.

Limitations and future Directions

As previously discussed, our cross sectional design limited us from observing changes in RAS which may occur with changes in PA and/or fitness. Our subjective
measure of PA via self-report questionnaire may not have provided an accurate representation of our participants’ level of PA. Future studies should employ a more objective measure of PA such as an accelerometer.

Future studies should also prospectively examining changes in fitness and RAS over time, and determine if an exercise intervention might affect RAS measurements in persons born PT with VLBW. Vigorous exercise should be incorporated, as it is most likely to improve fitness. 1

Conclusion

Our findings indicate that formerly PT/VLBW adolescents have a RAS imbalance demonstrated by a higher ratio of plasma Ang II:Ang 1-7 compared to their term-born peers. This relationship appears to be primarily due to lower levels of Ang 1-7. The lower aerobic fitness in PT/VLBW subjects may have partially contributed to this higher RAS ratio, as evidenced by our mediational analysis.

Decreased fitness has been shown to be associated with increased risk for future chronic disease including diabetes, hypertension, and cardiovascular disease. \(^{20}\) Additionally, a higher ratio of Ang II:Ang 1-7 has been shown to be associated with many of the same chronic diseases. \(^{16,92}\) Interventions targeting improvements in aerobic fitness through vigorous PA, may help to reduce the elevated Ang II: Ang 1-7 ratio and, ultimately, risk for chronic disease in this at risk PT/VLBW population.
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Curriculum Vitae

Education:

Wake Forest University: Master of Science in Health and Exercise Science, 2016.
  Teaching Assistantship
  Laboratory Coordinator

University of Delaware: Bachelor of Science in Exercise Science, 2014.
  Undergraduate Research Award: 2013
  Summer Scholars Scholarship: 2013
  NUCLEUS science program member

Presentations:

MARC-ACSM: Women's Health and Hypertension, 2013

Undergraduate Research Symposium: 2013

SEACSM: The Renin Angiotensin System and Blood Pressure in Preterm Very Low Birth Weight Adolescents, 2016

Organization Memberships:

American College of Sports Medicine