

**ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN THE
DEVELOPMENT OF HYPERTENSION AND METABOLIC
SYNDROME IN LOW BIRTH WEIGHT OFFSPRING**

By

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ABSTRACT

The sympathetic nervous system plays a pivotal role in the development of the metabolic syndrome particularly hypertension in low birth weight offspring. The Barker hypothesis reported that individuals born with low birth weight were at risk of developing chronic diseases later in life. Animal and human studies have since confirmed this relationship, however the mechanisms involved remain unknown. Therefore, this study investigated the role of the sympathetic nervous system in protein restricted rats and pre-clinical University of Zimbabwe students whose birth weights were less than 2.5kg. According to the Barker hypothesis adverse influences early in foetal development can result in permanent changes in the metabolism and physiology with such changes resulting in increased disease risk of the metabolic syndrome in adulthood. It was hypothesized that the sympathetic nervous system was the mechanism by which the metabolic syndrome particularly hypertension occurred in low birth weight and intrauterine growth restricted offspring.

An intrauterine growth restricted (IUGR) rat model was developed using local Zimbabweans diets. Using this model, mean blood pressure (mmHg) measured using the tail cuff method was significantly higher within 10 weeks in IUGR rats than in normal rats (122.46 ± 2.73 vs 106.50 ± 5.81 $p=0.0001$). The blood pressure significantly decreased in IUGR rats whose renal nerve (sympathetic influence) had been denervated (103.44 ± 8.17 mmHg vs 98.49 ± 7.85 mmHg $p=0.013$).

A series of human experiments were then conducted in young adults from the University of Zimbabwe. The students were put through exercise paces to allow the expression of the autonomic nervous system. Heart Rate Variability (HRV) ratio, and Pulse Wave Amplitude (PWA) responses to activation of the muscle metaboreflex were exaggerated in low birth weight (LBW) compared to normal birth weight (NBW) offspring (HRV: 1.015 ± 1.034 vs 0.119 ± 0.789 $p<0.05$) PWA: -1.320 ± 1.064 vs -0.735 ± 0.63 mV $p=0.28$. This suggested that the exercise pressor reflex, which is involved in tight regulation of the cardiovascular response to exercise, is persistently dysregulated into early adulthood for LBW individuals.

The study showed a significant difference in decrease in diastolic function (E/A ratio) in LBW than NBW groups (0.48 ± 0.27 vs 0.19 ± 0.18 $p=0.031$) and a significant association between LBW and exercise induced cardiac fatigue with diastolic dysfunction ($p<0.001$ odds risk ratio 7.5[95% CI 2.7-20.7]). Furthermore, the mechanisms of exercise induced cardiac fatigue involved transient myocardial stunning and a decrease in sensitivity of myocardium to catecholamines reducing the effect of adrenaline in improving both systolic and diastolic function during exercise.

LBW was associated with exercise induced hypertension (EIH) (62% vs 32% $p<0.05$) and post exercise hypotension (PEH) in young adults, a phenomenon hypothesised to be mediated by two processes; physiological down regulation of the cholinergic sympathetic vasodilator receptors in peripheral tissues due to chronic sympathetic stimulation; and increased wall thickening (EIH) and derangements in sympathetic tone (PEH). In the glucose tolerance study in LBW young adults had elevated sympathetic activity and were glucose intolerant (after 60mins 8.4 ± 0.56 mmol/l vs 7.57 ± 0.36 mmol/l $p=0.035$).

It is concluded that the sympathetic nervous system possibly has a role in the development of the metabolic syndrome in low birth weight humans and intrauterine growth restricted rats.

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PUBLICATIONS RELATED TO THE THESIS

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Conjoint Work with Supervisor and other persons

The work described in this thesis formed part of a collaborative project involving not only the Supervisors, Professor H.M. Chinyanga and Professor; J. Mufunda but also Professor. Z.A.R Gomo. Because of the nature of the collaborative work that Jephath Chifamba was the initiator and driver, it is necessary to include within the thesis (as permitted under regulation 11-1-7) some data, which was not obtained by Mr. Jephath Chifamba personally.

Professor H.M, Chinyanga (Supervisor):

Mr. J Chifamba (candidate):

Dedicated to

Kudzanai my sweetheart, Michelle (Mimi) and Tinomutenda (TJ)

In memory of

Martha Chifamba

(My mum)

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 COMMUNICABLE AND NON-COMMUNICABLE DISEASE BURDENS

1.1.1 Sub-Saharan Africa and non-communicable disease

The first half of the last century (1900s) has seen a five-fold increase in cardiovascular diseases (CVD) in most Western countries, followed by a partial fall in the second half of the century (WHO, 2015). The increase was largely a consequence of the health transitions secondary to urbanization. The health transitions were characterized by decreases in routine usual physical activity (e.g walking, manual labour) and increased energy intake (maladaptation to urbanisation) (Teo *et al.*, 2009). The subsequent fall in CVD occurring in some regions of the developed world, is partly related to the progressive adaptation of lifestyles to counteract the adverse effects of urbanization, increasing leisure time activity, modifying dietary patterns, and better management of risk factors (Valentin & Kelly, 2010).

Developing countries are now undergoing several transitions which collectively affect health, resulting in decreases in childhood mortality and infectious diseases (Teo *et al.*, 2009), but with a concomitant increase in chronic illness, in particular CVD and diabetes mellitus (DM) (WHO, 2015). These shifts are most marked in urban areas, which form a “macro environment” of rapid and pronounced change relative to rural

areas. The changes include a shift in food intake (processed food from local natural) and decreased energy expenditure jointly leading to obesity (Teo *et al.*, 2009). Increased psychosocial pressures and breakdown of traditional support systems (family ties - extended families) may affect susceptibility of individuals to chronic diseases, including CVD (Reddy & Yusuf, 1998). Recently Chinyanga and Halahala (2014) added another possible risk factor when they observed that the mode of delivery (vaginal or caesarean section) predisposed the offspring to exercise induced hypertension.

The emergence of CVD in developing countries was highlighted in a review fifteen years ago (Reddy & Yusuf, 1998). It remains an important public health issue and lends itself to many opportunities for research. The World Health Organization, in its 1998 report, stated that “in developing countries, as their economies grow, non-communicable diseases will become more prevalent, largely due to the adoption of “western” lifestyles and their accompanying risk factors such as smoking, high-fat diet, lack of exercise” (WHO, 1998; Cooper & Bovet, 2013). In absolute terms, developing countries contribute more to the global burden of CVD than the developed countries (Lopez *et al.*, 2006).

The World Health Organization Regional Office for Africa (WHO, 2011; WHO-AFRO, 2014) commissioned a study to compile and analyse published reports on non-communicable diseases (NCDs) in Africa to build evidence on the burden of NCDs in the region. Anecdotally, little information or literature was available on the subject. The

purpose of the WHO (2011) study was to establish the status of NCDs in Africa by using published sources of information. A literature search was done through MEDLINE/Pub Med and Google to identify studies that reported on prevalence rates of NCD risk factors. The study confirmed that information on NCDs in Africa was lacking (Cooper & Bovet, 2013; WHO-AFRO, 2014). In available published studies, the prevalence of hypertension was found to be rapidly increasing, from 3% in rural areas to > 30% in some urban settings. In some populations, hypertension prevalence rates were higher in women than in men while the opposite was true in others. Most people with hypertension were not aware of their condition, and of those who were on treatment, less than 20% had optimal control. The prevalence of diabetes mirrored that of hypertension in the same period, from less than 1% in some rural areas to more than 20% in some selected populations and racial groupings in urban settings. The predominant type was type 2 diabetes mellitus, which accounted for more than 80% of all cases in some reports and tended to present later in life. The prevalence of tobacco smoking also varied across the continent, from less 1% in rural women to 50% in some urban men.

The WHO NCDs 2008 Country report for people under 70 years showed cardiovascular diseases were responsible for the largest proportion of NCD deaths (39%) (WHO-AFRO, 2014). Rapid urbanisation with changes in lifestyle, especially dietary habits and physical activity patterns have been suggested to at least partially explain the increased prevalence of cardiovascular diseases. (Iqbal *et al.*, 2008; Teo *et al.*, 2013; Lear *et al.*, 2014). Three transitions, epidemiological, nutritional, and social are proposed to be responsible for the increases in non-communicable

diseases in developing countries. Lack of awareness among urban and rural dwellers (Chow *et al.*, 2013), urban poor living conditions, under nutrition and increased psychosocial stress over a prolonged period have been reported to have a role in these transitions (Cooper & Bovet, 2013). Another dimension to the empirical understanding of the risk factors for the metabolic syndrome (hypertension, insulin resistance, obesity and dyslipidemia) associated with urbanization has been added and that is “foetal programming” (Alexander, 2006). As such adult life style factors and genetics have for years been the focus of research for the aetiology of metabolic syndrome particularly hypertension. However, intra-uterine factors have also become important (Barker, 1995a, 1995b). Foetal programming has been realized to be a major risk factor for cardiovascular disease in almost the same manner as “bad luck” is invoked for cancer (Couzin-Frankel, 2015).

Communicable diseases such as HIV/AIDS, tuberculosis and malaria remain the main causes of premature death in sub-Saharan Africa and the rest of the world (Lopez *et al.*, 2006; Mboera *et al.*, 2014; Murray *et al.*, 2014). However, non-communicable diseases such as cardiovascular diseases, and diabetes mellitus as mentioned earlier, are becoming increasingly prevalent with the growth of the proportion of urban population. Nsakashalo-Senkwe *et al.* (2011) reported a prevalence of impaired glucose tolerance or diabetes mellitus of 4% in Zambia, furthermore, Brown *et al.* (2014) reported that their data showed that diabetes mellitus consumed more time and medicines in sub-Saharan African refuting the common thought that it caused minor problems to sub-Saharan health systems. The cost of primary prevention of cardiovascular diseases in Tanzania using the WHO's absolute risk approach

guidelines ranged from USD\$118 per annum to USD\$127 dollars per annum per person (Ngalesoni *et al*, 2014). Non-communicable diseases contributed more to the double burden of diseases the country was facing. In support Goma *et al.* (2011) reported a hypertension prevalence of 34.8% (38% in males and 33.3% in females) in Zambia. In Mozambique non-communicable diseases accounted for 28% deaths (Silva-Matos & Beran, 2012). Silva-Matos and Beran (2012) recommended education and social changes to promote healthier life styles. Without going through all the sub-Saharan countries reports which number more than 1 050 in the last five years (Pub med), a snap shot of these studies clearly show that non-communicable diseases are increasingly becoming a major public health issue if not already in these developing countries. Woelk (1995) in a review article reported that this increase in hypertension in the continent could also, at least, be partly explained by the increase in low birth weight offspring.

1.1.2 Non-Communicable diseases and hypertension in Zimbabwe

Hypertension was commonly seen among African Zimbabweans by Michael Gelfand when he stated started his medical practice in the 1940's, although it did not predispose to coronary heart diseases (Fallon & Enig, 1999). In the same article he is quoted to have reported an increase in diabetes mellitus as a result of increased consumption of western foods such as white bread, refined sugar, jam and tea among the Zimbabwean Africans. Indeed Mufunda *et al.* (2000) reported the prevalence of hypertension in urban Zimbabwe to be 29% in women and 24% in men. More recently as a result of the strained health system, an increase in mortality related to both

communicable and non-communicable diseases was reported at Mpilo Central Hospital in Bulawayo (Bardgett *et al.*, 2006). A decade earlier, the absence of prevalence studies that could be easily accessed and allow policy formulation and resource utilisation were absent as a result hospital data records between 1990 and 1997 were used to generate prevalence records (Mufunda *et al.*, 2006). Mufunda *et al.* (2006) noted that hypertension prevalence rates increased four times, diabetes increased 3.7 times and cerebrovascular accidents increased 3 times during the period of the study. It was shown in a National Survey of Zimbabwe Non-Communicable Disease Risk Factors (Hakim *et al.*, 2005) that hypertension prevalence was 23.2% in males and 29% in females agreeing with previous findings (Mufunda *et al.*, 2000). The same report mentioned the prevalence of diabetes nationally averaged approximately 4%, hypercholesterolemia prevalence was 3.2% males and 4.7% females (Hakim *et al.*, 2005).

It is proposed for Zimbabwe that the increase in non-communicable disease burden, particularly cardiovascular disease whose major risk factor is hypertension is a result of the three important transitions the country is going through. These transitions have an important impact on a fourth transition (health). These transitions are shown in Table 1 (Reddy & Yusuf, 1998; Yusuf *et al.*, 2001; Teo *et al.*, 2009).

Table 1: Three important cardiovascular transitions Zimbabwe is going through

ECONOMIC TRANSITION		
<ul style="list-style-type: none"> • Largely agrarian • Limited industrial base 	→	<ul style="list-style-type: none"> • Globalisation of the economy • Greater 'urban' opportunities • Agricultural subsidies under threat • Rural to urban migration • Enhanced rich-poor divide
NUTRITION TRANSITION		
<ul style="list-style-type: none"> • Importance of the 'staple' in the diet • Vegetarianism • Low fat intakes 	→	<ul style="list-style-type: none"> • Increased variety in diets • Increased fat intake • Increased refined sugars • Dual problems of obesity and adult under nutrition-leading to low birth weight
LIFESTYLE / BEHAVIOURAL TRANSITION		
<ul style="list-style-type: none"> • Joint families / support systems 	→	<ul style="list-style-type: none"> • Nuclear families • Increased smoking • Decreased physical activity • Increases job stress • Decreased social support structures • Financial stresses and financial demands
HEALTH / EPIDEMIOLOGICAL TRANSITION		

Communicable diseases (Although they still remain a public health challenge in Zimbabwe)	→	Non-communicable diseases including cardiovascular disease
---------------------------------------------------------------------------------------------	---	------------------------------------------------------------

Overall, it is estimated that in the coming decades, chronic diseases of aging will replace infectious diseases as the most common disease cluster (Yusuf *et al.*, 2001). Of the chronic diseases, cardiovascular disease is projected to assume primacy in terms of persons affected, health expenditure, impact on productivity and health policy (Yusuf *et al.*, 2001; Valentin & Kelly, 2010). In order to effectively intervene and impact on these projected outcomes, it is imperative that the risk factors for cardiovascular disease, both traditional and unique are delineated.

Studies have shown that CVD and type 2 diabetes mellitus are diseases of adult lifestyle. Their risk factors include bad diets with high-energy and high-fat diets, smoking and a lack of physical exercise, which results in obesity, hypertension, dyslipidaemia and other metabolic changes (e.g., increased pro-inflammatory cytokines). Research findings have also shown that these risk factors are not the full story. The basis for this study relates to another possible risk factor (low birth weight) whose prevalence is increasing and as Woelk (1995) reported is a risk factor that is not receiving adequate attention but which can be easily controlled and thus reduce the spiralling cardiovascular disease prevalence.

1.1.3 Why low birth weight could be an important risk factor

Firstly, public health control of disease that is targeted at adult nutrition and behaviours maybe insufficient to control the advent of non-communicable diseases in Zimbabwe and other sub-Saharan African countries (McMullen *et al.*, 2012). This may be so because current studies demonstrate that the problem is starting much earlier than previously thought and primary preventive methods targeted at adults only may not produce the reduction targets that are projected without going back to the conditions found in the uterine environment. Barker *et al.* (1989) reported in a British sample study of 9 921 ten year olds and 3 259 adults that systolic blood pressure was inversely related to birth weight. They also noted that the association was independent of gestational age and could therefore be attributed to reduced foetal growth. The conclusion was that intrauterine environment influenced blood pressure during adult life. It is important to mention that there are many who dispute these findings (Paneth *et al.*, 1996; Leon, 1998; Baird *et al.*, 2001), the latest being Zohdi *et al.* (2014) in their review in which they give possible reasons for the observed differences.

Woelk *et al.* (1998) made the first report of a relationship between blood pressure and low birth weight among children in Harare, Zimbabwe. They found that systolic blood pressure was inversely related to birth weight among 756 school children (mean age 6.5 years). They however noted that diastolic blood pressure was not associated with any intrauterine indices. Chifamba *et al.* (2004) reported similar findings but also noted that low birth weight children retained sodium and excreted more potassium compared to normal birth weight children.

The incidence of low birth weight (defined as birth weight <2.5 Kg) is increasing in Zimbabwe. This is despite a down turn in the number of adults and children admitted to hospital suffering from severe malnutrition 17 years ago (Ministry of Health and Child Welfare 1998). Recent reports attribute this increase to variable weather (Paul *et al.*, 2011; Gwatirisa & Manderson, 2012). Health survey figures show that the number of infants weighing less than 2.5 Kg at birth has increased from 8.6% in 1992 to 13.8% in 1998, which represents over 35,000 births and is estimated at 11% 2013 (UNICEF, 2014; UNICEF 2015). Clearly, this has important implications for future health care provision. The increased prevalence of HIV infection in Zimbabwe is also likely to be the cause of increased low birth weight (Ikeogu *et al.*, 1997; Mason *et al.*, 2010; Prendergast *et al.*, 2011). A report from Malawi, reported that Maternal HIV infection was associated with reduced birth weight (Kalanda *et al.*, 2005). Finding the key mechanisms that might allow for successful intervention is important if developing countries like Zimbabwe are to mitigate against another epidemic, this time of non-communicable diseases which according to the WHO Country report accounted for 21% of all deaths in Zimbabwe in 2008.

1. 1.4 Pathogenesis of hypertension: investigator's research journey

The early days of the investigator's research focused on what was labelled as "urbanization related hypertension". The studies were mainly case control studies that compared urban and rural hypertensive and normotensives subjects (Mufunda *et al.*, 1993b). Although the subjects demonstrated salt sensitivity (blood pressure increased

with increased sodium intake), it was reported that there was no relationship between salt sensitivity and fasting blood insulin levels. Some of the results pointed towards kidney problems in the black populations and investigators ventured into measuring microalbuminuria which was characteristic of the hypertensive subjects (Hwang *et al.*, 2000). The prevalence of hypertension was reported to be high in urban compared to rural subjects and increase in body mass index in both males and females was a risk factor for this increase (Mufunda *et al.*, 2000). Insulin resistance was involved in urbanization-related hypertension (Mufunda *et al.*, 1994).

Sever (1995) published an article in which he described hypertension as a disease of multifactorial origin with evidence of environmental factors implicated in its aetiology. Sever (1995) reported that stress, obesity, dietary sodium several genetic abnormalities predisposed individuals to hypertension. The results of this would be heterogeneity in response to antihypertensive treatment, with one drug unlikely to control blood pressure in more than 25-50% of hypertensives. He also concluded that this was the rationale for combination therapies. The investigator's research group was left with many unanswered questions those early years, which in retrospect it can be noted that the birth weight of the subjects was not considered in any of the studies.

Faced with numerous options to consider as research focus routes and with the painful agenda of jumping from one area of investigation to another the investigator visited Manchester medical school for contact leave and met Dr. N. Ashton who was the host. His focus area of research was in prenatal development and hypertension in

the adult offspring (Ashton, 2000; Gouldsborough & Ashton, 2001; Sahajpal & Ashton, 2003) .This was almost a eureka moment in that this was likely to be the missing variable in pathogenesis on hypertension studies. Suddenly I got exposed to the theories that were floating around at the time that intrauterine insults were associated with the development of metabolic and cardiovascular diseases in adulthood (Barker & Martyn, 1992).

In previous case control studies conducted, it became clear that birth weight of the subjects, a matter that greatly influenced their blood pressures in adulthood, was not considered. It also became plausible that the elevated blood pressures recorded in the United States hypertensive blacks compared to whites could have been higher because this 'missing variable 'was not included in the analysis. Reports showed that during the times of some of the USA studies blacks lived in poor conditions and the prevalence of low birth weight was high among the blacks. Lackland *et al.* (2002) reported that their findings indicated that birth weight was associated with use of calcium channel antagonists in black women and angiotensin converting enzyme inhibitors in white men. Could it be possible that the differences observed in Zimbabwean blacks migrating from rural to urban areas and then responding differently to the environmental insults could have been a result of prenatal programming.

1.2 MALNUTRITION AND LOW BIRTH WEIGHT

Malnutrition and micronutrient deficiency are important public health issues in developing countries like Zimbabwe (De Onis *et al.*, 2004; Müller & Krawinkel, 2005; Olivieri *et al.*, 2008) . Malnutrition results in offspring born with low birth weight (LBW) (2.5kgs in humans) (Delisle, 2002) . Beside malnutrition other risk factors for LBW include young age mothers, previous LBW infants, hypertension (Romundstad *et al.*, 2007) or cardiovascular diseases, multiple pregnancies, drug addiction, alcohol abuse, and insufficient prenatal care. Environmental risk factors such as smoking, lead exposure, and other air pollutants are also risk factors for LBW (Delisle, 2002). In this investigation, low birth weight in the young adults could have been caused by any of the risk factors above. In the animal studies the low birth weight was obtained by dietary protein restriction. In both cases this study assumes low birth weight as a consequence of intrauterine growth restriction (IUGR).

1.3 ORIGINS OF LOW BIRTH WEIGHT STUDIES

In humans McDonald reported the relationship between low birth weight and future life events as far back as 1964. He conducted three studies on deafness (McDonald 1964a), fits (McDonald 1964b) and intelligence (McDonald 1964c) in children with very low birth weight. In the intelligence study he reported that intellectual damage did not occur in their study of intelligence in children of very low birth weight. The study was addressing conflicting reports about intelligence in children of very low birth weight. Rose (1964) reported that siblings of those who died as infants or had still birth experienced twice the rate of ischemic heart diseases than in control groups.

Forsdahl (1977) reported a geographical correlation among Norwegians between coronary heart disease (CHD) mortality in 1964–67 and infant mortality rates 70 years earlier (1896–1925). Fall (2013) reported that growing up in poverty areas caused ‘permanent damage’ perhaps due to a ‘nutritional deficit’, which resulted in ‘life-long vulnerability’ to an affluent adult lifestyle.

In 1989, David Barker a physician and researcher from the United Kingdom (Southampton University) coined the term foetal programming after discovering the relationship between low birth weight and lifetime risk of coronary heart diseases (Barker *et al*, 1989). In a British study of 9 921 ten year olds and 3 259 adults, systolic blood pressure was significantly inversely related to birth weight. They reported that the association was independent of gestational age and may therefore be attributed to reduced foetal growth. Barker *et al.* (1989) concluded that the intrauterine environment influenced blood pressure during adult life. Since then others have confirmed Barker *et al.*’s (1989) findings. Indeed, numerous studies in Europe, the United States and to lesser extent developing countries such as India have confirmed Barker’s findings of an association between low birth weight and adult high blood pressure. Meta-analysis of 34 separate studies with a total sample size of over 66,000 demonstrated an increase in blood pressure of 3.5 mmHg for every Kg decrease in birth weight (Law & Shiell, 1996). Many of the early studies were reviewed by Delisle (2002). Table 2 shows a selection of another set of studies up to 2014. A list of those that do not confirm the Barker hypothesis studies is provided in Table 3.

Table 2: A selection of studies that confirmed Barker *et al.*'s (1989) findings sorted by date. The outcomes for blood pressure, insulin levels, lipid levels.

Reference	Study type	Findings
Zhang <i>et al.</i> (2014)	USA national health survey of children and adolescents 6-15	LBW was inversely associated with SBP (in girls) fasting insulin and HOMA-homeostatic model assessment
Mori <i>et al.</i> (2012)	243 healthy Japanese high school girls aged 16.4 ± 1.4	Low birth weight was associated with risks of Metabolic syndrome (SBP, DBP, TG, insulin level, and insulin resistance)
Kikuchi & Uchiyama (2010)	Describe several epidemiological studies performed in Japan and discuss whether Developmental Origins of Health and Disease (DOHaD) is applicable to children in present day Japan	It was found that systolic blood pressure; total cholesterol and adiponectin were associated with birth weight. Hyperinsulinemia, high blood pressure, elevated transaminase levels and prevalence of metabolic syndrome in obese children were inversely correlated with birth weight and positively correlated with current weight and waist circumference
Boyne <i>et al.</i> (2010)	The Jamaican Vulnerable Windows Cohort is a longitudinal survey of 569 mothers and their offspring recruited from the first trimester.	Systolic blood pressure and fasting glucose concentration were inversely related to birth weight in boys but directly associated in girls
Gamborg <i>et al.</i> (2007)	The authors investigated the shape, sex- and age-dependency, and possible confounding of the association between birth weight and systolic blood pressure (SBP) in 197,954 adults from 20 Nordic cohorts (birth years 1910-1987), one of which	The results showed that the meta-analysis supported the evidence of an inverse birth weight-SBP association, regardless of adjustment for concurrent body size.

	included 166,249 Swedish male conscripts	
Osmond <i>et al.</i> (2007)	12 439 people born in Helsinki during 1934 to 1944	Thinness during infancy is associated with an increased risk of stroke in later life
Veena <i>et al.</i> (2007)	Offspring (20-46 years) of men and women who had been born in Holdsworth Memorial Hospital, Mysore, India.	Lower birth weight was associated with an increased risk of glucose intolerance (impaired glucose tolerance, impaired fasting glucose or type 2 diabetes) and higher cholesterol and triacylglycerol concentrations ($p < 0.05$ for all adjusted for sex and age)
Stein <i>et al.</i> (2006)	Individuals (mean age 59 y) born in western Holland between January 1945 and March 1946 (mothers exposed to the Dutch Famine before or during gestation; $n = 359$)	Birth weight was inversely related to systolic (-4.14 mmHg per kg; 95% confidence interval (CI) -7.24, -1.03; $p < 0.01$) and diastolic (-2.09 mmHg per kg; 95% CI -3.77, -0.41; $p < 0.05$) blood pressure and to the prevalence of hypertension (odds ratio 0.67 per kg, 95% CI: 0.49, 0.93) (all age- and sex-adjusted)
Tian <i>et al.</i> (2006)	973 individuals from a population-based cross-sectional survey in Shanghai, China	Birth weight is inversely associated with the risk of type 2 diabetes
Kim <i>et al.</i> (2006)	An at-home questionnaire survey was performed on 660 middle school students (12-15 years) in Seoul, Korea, and 152 cases were randomly selected based on their birth weight	Low birth weight may predict the risk of the insulin resistance and its progression over age
Ramadhani <i>et al.</i> (2006)	Seven hundred and forty-four young adults aged 26-31 years participated in the ARYA birth cohort. Birth characteristics were available from charts	Birth weight was inversely related to systolic blood pressure (linear regression coefficient, -1.9 mmHg/kg birth weight; 95% CI -3.4 to -0.3) and to (log) triglycerides in mmol/L (-0.03/kg birth weight; 95% CI -0.06 to -

	kept by the Municipal Health Service, Utrecht, The Netherlands	0.01), adjusted for gender, current body mass index and current education
Schack-Nielsen <i>et al</i> (2002)	Case-cohort study of obesity in Danish men	The birth weight was inversely related to systolic blood pressure, even when controlled for BMI since the age of 7 years
Gunnarsdottir <i>et al.</i> (2002)	The study included 4601 men and women born 1914-1935 in Reykjavik, Iceland, who participated in the Reykjavik Study of the Icelandic Heart Association	Birth weight was inversely related to hypertension in adulthood in women (P for trend < 0.001). The relationship was of borderline significance in men (P for trend = 0.051). A low ponderal index was significantly associated with high BP in women (P for trend = 0.025) but not men (P > 0.05). They also concluded The results support the hypothesis that the association between birth weight and hypertension is not of genetic origin only
Andersson <i>et al.</i> (2000)	Original midwife records of 438 women born at term participating in a prospective population study in Göteborg, Sweden with blood pressure and hypertension assessment at both 50 and 60 years of age.	Systolic blood pressure at both age levels showed a U-shaped relationship to weight and length at birth. Size at birth was a predictor of hypertension risk in women at 60 years but not 50 years
Huxley <i>et al.</i> (2000)	All papers published between March 1996 and March 2000 that examined the relationship between birth weight and systolic blood pressure were identified and combined with the papers examined in a previous review. More than 444,000 male and female subjects aged 0-84 years of all ages and races.	Eighty studies described the relationship of blood pressure with birth weight The majority of the studies in children, adolescents and adults reported that blood pressure fell with increasing birth weight, the size of the effect being approximately 2 mmHg/kg.

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Table 3: Shows a selection of those studies that do not support the Barker hypothesis

<p>Daly <i>et al.</i> (2005)</p>	<p>A retrospective cohort with birth weight collected from hospital records of 855 (68%) out of 1260 Auckland-born students who had blood pressure, fasting blood lipids, and glucose measured while in Year 11-13 at high school-New Zealand</p>	<p>After controlling for sex, age, and ethnicity, none of the following cardiovascular risk factors were associated with birth weight ($p > 0.05$): systolic and diastolic blood pressure, total cholesterol, HDL cholesterol, triglycerides, and glucose. The conclusion was results do not support for the 'foetal origins' hypothesis</p>
<p>Williams & Poulton (2002)</p>	<p>Data from a cohort of 891 infants born in Dunedin, New Zealand, in 1972-1973 whose blood pressure was measured at 2-year intervals from age 7 years to age 15 years and at ages 18 and 26 years were used to test this hypothesis.</p>	<p>Three regression models based on standardized scores for weight and height were used. The first showed that at any of the ages at which the cohort was assessed, an increase in birth weight of one z score (one standard deviation) was commensurate with a decrease of 0.29 mmHg (95% confidence interval: -0.17, 0.76) in blood pressure. The second model showed that a one-z-score increase in weight between birth and a subsequent age was associated with an increase in systolic blood pressure of 0.96 mmHg (95% confidence interval: 0.53, 1.38). They concluded results fail to</p>

		support the foetal origins hypothesis
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Kikuchi & Uchiyama (2010) described several Japanese studies that had investigated the “developmental origins of adult health and disease” in that country and found that the studies supported the Barker hypothesis, concluding that the concept was relevant in Japan so Paediatricians and schoolteachers should therefore understand it, so that they can provide information to children and their families regarding appropriate diet to reduce the likelihood of developing adult diseases in later life. Stein *et al.* (2006) in the famous Dutch famine studies concluded that, exposure to famine during gestation may predispose to the development of hypertension in middle age.

This scientific field of biomedical and public health has been named Developmental Origins of Health and Disease (DOHaD). Although the concept developed two decades ago, lack of interest in integrative sciences such as physiology overlooked it in the later part of the 20th century with preference to the dominant molecular biology. There are other reasons listed by Hanson & Gluckman (2014) which include, lack of a conceptual framework, confusion between factors correlated with disease risk and those involved in causation, assumption of operation of a single pathway, undue focus on low birth weight, lack of plausible underlying biological mechanisms, failure to recognize its importance under normal rather than only under extreme conditions, lack of evidence of its relative importance in relation to other risk factors and lack of plausible ways to use the concept clinically and in public health.

In spite of these gaps, the International Society for Developmental Origins of Health and Disease was set up to promote research into the foetal and developmental origins of disease and involves scientists from many backgrounds (DOHaD, 2015a) and a dedicated journal was started with its first publication in 2009 (DOHaD, 2015b).

The majority of evidence which supports the concept of DOHaD comes from animal studies, but there is increasing evidence from human physiology research (Hanson & Gluckman, 2014).

David Barker originated the term foetal programming and since then Lucas (1991) was the first to define it. He defined it as a more general process whereby a stimulus or insult at a critical period of development has lasting or lifelong significance. During this time, a stimulus or insult may precipitate permanent programming of metabolic, cardiovascular and endocrine pathways. Hanson & Gluckman (2014) have recently proposed that what has been called programming in the last two decades should be called 'conditioning'. Their assertion is that programming implies a ballistic progression of development that uterine events will result in disease no matter what. They believe conditioning maintains the levels of plasticity that with some behavioural modification will not necessarily result in all the uterine growth restricted offspring developing the programmed" diseases. All the results including those of Barker *et al.* (1989) do show that not all growth restricted individuals developed disease. In this study the term foetal programming will however be used.

Whichever terminology DOHaD investigators will agree on, the phenomenon of foetal environments influencing development of offspring is not new in biology. In the crocodiles (and other amniote vertebrates that are classified under the reptile class (Warner & Shine, 2008) temperatures experienced during embryonic development determine the sex of the offspring (Lance, 2009). If the temperature is cool, around 30 degrees C, the hatchlings are all female. Warmer temperatures, around 34 degrees C, hatch all males. Bee queen larva develop differently because it is fed on royal jelly, a protein-rich secretion from glands on the heads of young workers (Shi *et al.*, 2011). If the bee larva are not fed royal jelly they develop into a regular worker bee (Winston, 1991). This comparison however does not fit well because in humans a 'memory factor' is present (disease phenotype appears many years later) while the changes are progressive from early life (no silent delay or memory time) in the crocodile or bee cases.

There are several proposals on how intrauterine growth restriction (represented by low birth weight) can predispose to hypertension. Hypoxia and foetal malnutrition arising in an adverse intra-uterine environment tend to increase foetal vascular resistance, inhibits angiogenesis and increases resting sympathetic discharge thus increasing peripheral arteriolar resistance thus predisposing to hypertension. Stress on the foetus due to restricted growth increases the circulating levels of cortisol and other glucocorticoids and such excessive activity of glucocorticoids leads to an abnormal hypothalamic-pituitary-adrenal axis potentiating hypertension. Intra-uterine growth

restriction may lead to remodelled and less efficient hearts in childhood. The development of essential hypertension in adult life might be due to decreased glomerular filtration surface area in kidneys with a reduced number of nephrons developed in an adverse utero. In all these the sympathetic nervous system is proposed in this study to have a central role.

1.4 POSSIBLE MECHANISMS OF LBW PROGRAMMED HYPERTENSION IN ADULT OFFSPRING

Many epidemiological studies have provided a basis for experimental studies of hypertension and cardiovascular disease seen in adults who were born with low birth weight. The main systems involved in developmental programming of hypertension include the renal system, the neuroendocrine systems and the vascular system (Ligi *et al.*, 2010).

1.4.1 Number of nephrons among LBW

In a novel perspective on the origins of essential hypertension by Brenner *et al.* (1988) (quoted by Mackenzie & Brenner (1995)) they advanced a notion that the number of nephrons at birth were inversely related to the risk of developing hypertension in adulthood. It is generally understood that the kidney plays an important role in the development and maintenance of essential hypertension by preventing the pressure natriuresis (Mackenzie *et al.*, 1995; Luyckx *et al.*, 2011). Guyton *et al.* (1984) highlighted the supremacy of the kidney in regulating the intravascular fluid so regulating long term blood pressure. Mackenzie *et al.* (1995) developed what they

described as the nephron number hypothesis, where they argued the point that the total nephron number was the overlooked determinant of the link between the pathogenesis of hypertension and the kidney. With the advent of the Barker *et al* (1989) hypothesis that low birth weight was associated with higher blood pressure in adult life, the hypertension observed in adults who were born low birth weight became an obvious call that the reduced number of nephrons seen in low birth weight individuals was responsible.

In salt sensitivity studies conducted by Mufunda *et al.* (Mufunda *et al.*, 1993b, 1998), controlling for birth weight would have revealed a different set of results. Salt sensitivity inversely correlated with kidney length and reduced renal mass in growth-restricted children who possessed a risk for a lower renal function and increased salt sensitivity (de Boer *et al.*, 2008; Simonetti *et al.*, 2008).

1.4.2 Role of glucocorticoids

Maternal nutrient restriction during pregnancy predisposes offspring to metabolic alterations (Óvilo *et al.*, 2014). Humans and animals have a gradient of active glucocorticoid levels across the placenta. The foetus is protected from the excess of corticosteroids in maternal circulation by an enzyme in the placenta, an isoform of 11 β -hydroxysteroiddehydrogenase (Benediktsson *et al.*, 1993; Welberg *et al.*, 2005; Börzsönyi *et al.*, 2012). This protective enzyme converts glucocorticoids to inactive forms, thereby ensuring the autonomy of the foetal hypothalamic-pituitary-adrenal axis and preventing glucocorticoid activation of genes that promote early maturation of

tissues dehydrogenase (Benediktsson *et al.*, 1993; Welberg *et al.*, 2005; Börzsönyi *et al.*, 2012;). Studies of rats fed low-protein diets in pregnancy have shown that placental 1 β -hydroxysteroid dehydrogenase is down-regulated by under nutrition (Langley-Evans *et al.*, 1996a, 1996b) and similar effects are noted in nutrient restricted sheep (McMullen *et al.*, 2004).

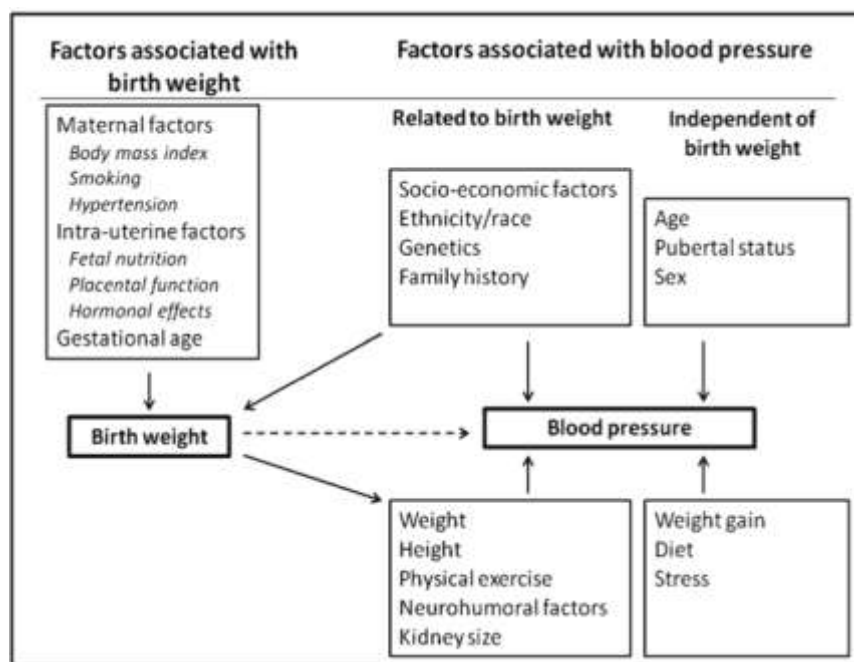


Figure 1: Simplified overview of factors affecting the complex relationship between birth weight and blood pressure (Edvardsson *et al.*, 2012)

1.4.3 Tissue remodelling

Nutritional restriction programmes long-term function of organs by altering their structure as shown in Zohdi *et al.* (2014). Body organs originate from embryonic progenitor cells. The few cells go through developmental changes that allow them to increase in numbers (proliferate) and differentiate to specialised cells found in organs.

Adverse changes in nutrition at any point during the development will result in structural changes in that will results in a remodelled organ.

The above change which may reduce the number of cells will affect the function of the organ such as cell signalling and the resultant type of humoral factors secreted. The reduce number of cells will also affect the functional capacity of the particular organ. (Langley-Evans, 2009, 2013, 2015). Recent data has suggested that 11 β -hydroxysteroid dehydrogenase beside its role in the placenta, levels within the blood vessel wall influences vascular remodelling and angiogenesis (Walker, 2006).

1.4.4 Glucose intolerance and insulin resistance

Manipulating maternal nutritional load has been shown to affect glucose metabolism. In these studies it was shown that at a specific period of development during fetal growth (prenatal) or and suckling periods impacted the offspring in its glucose metabolism. These studies observed that restricting maternal protein diet resulted in offspring that had enhance glucose response in young animals and insulin resistance would develop in the older animals (Ozanne *et al.*, 2003; Eyzaguirre *et al.*, 2012; Thomas *et al.*, 2012; Coutinho *et al.*, 2013). Other studies have since shown that dams fed with high fat diets produced offspring that were programmed to have insulin resistance when older (Khan *et al.*, 2005; Armitage *et al.*, 2005). Iron deficiency during prenatal growth has not been shown to result in glucose, implying that specific nutrients are involved in programming cardiovascular diseases (Gambling *et al.*, 2003; Hossain *et al.*, 2011).

1.4.5 DNA methylation and epigenetic programming

Studies have shown that gene expression is modified by epigenetic mechanisms that include DNA methylation or histone acetylation. It has been shown that during fetal development, this is the time when specific patterns of DNA methylation are established. The pattern of methylation once established at this point is thought not to change and maternal nutritional insults of the fetal nutrition has permanent changes of gene expression of different organ cells (Young & Beaujean, 2004).

DNA methylation is catalysed by the methyltransferases which utilizes S-adenosylmethionine as the main methyl donor. DNA methylation is linked biochemical pathways such as the folate cycle and the methionine–homocysteine cycle (Kim *et al.*, 2009). Low-protein diet studies in pregnant dams have used casein as the major protein source (soya will also be used in this study), and amino acid methionine has been added as a supplement to provide requirements for sulphur. This results in changes in the methionine–homocysteine cycle that impact upon the provision of methyl donors for methylation of DNA. The view is supported by evidence from studies in which the low protein diet is supplemented with folic acid (Lillycrop *et al.*, 2005), or with glycine (Jackson *et al.*, 2002). These generally show that the effects of low-protein feeding can be reversed. Lillycrop *et al.* (2005), have shown that low protein feeding impacted upon both the expression and methylation status of specific genes (Lillycrop *et al.*, 2007; Burdge *et al.*, 2007). However, in contrast Bogdarina *et al.* (2004) found no evidence that maternal protein restriction impacted on the methylation of the promoter for hepatic glucokinase, despite programmed changes in gene expression.

Since McCance's (1962) studies which demonstrated long term effects of early nutrition in rats, numerous other animal studies have since shown that nutrition in early life can have long term effects on metabolism, growth, and neurodevelopment.

He also showed that this early nutrition could have effects on diseases such as hypertension, diabetes, atherosclerosis, and obesity.

The association between hypertension and low birth weight has been reported in experiments using rat models (Langley-Evans *et al.*, 1996c; Manning & Vehaskari, 2001; Vehaskari *et al.*, 2001).

Mechanistic pathways although divided into three major systems are multiple and still undefined and unclear. Ligi *et al.* (2010) report that the vascular structure and function play an important role, and in this investigation the sympathetic nerve activity has been suggested as the link.

1.5 THE FIGHT OR FLIGHT MODE

1.5.1 Metaboreflex

The muscle metaboreflex, sometimes called the muscle chemoreflex, is one of the principal mediators of the cardiovascular responses to exercise. The reflex is thought to be triggered by metabolic by-products of ischemia that stimulate afferent nerve endings in skeletal muscle and leads to an increase in sympathetic activity and cardiac output, and vasoconstriction in non-active tissues. Static/isometric handgrip exercise evokes an increase in heart rate, mean arterial pressure, muscle and skin sympathetic nervous activity. Blocking forearm blood circulation just before cessation of the exercise causes blood pressure to remain above resting levels (IJzerman *et al.*, 2003; Watanabe *et al.*, 2010). These responses are evoked by two neural mechanisms, components of the Muscle Metaboreflex, which are: central command

(neural signals of central origin) and the Exercise Pressor Reflex (EPR-a reflex arising from exercising muscle) (Murphy *et al.*, 2011). The central command has a minor role in cardiovascular regulation during exercise. The exercise pressor reflex is a feedback system arising from thinly myelinated mechanosensitive (group III/A δ -fibers) and unmyelinated metabosensitive (group IV/C-fibers) afferents in the skeletal muscle (Murphy *et al.*, 2011). When oxygen delivery to active skeletal muscle is insufficient to meet the metabolic demands, metabolites e.g. lactic acid, adenosine, diprotonated phosphate, potassium, H⁺ and arachidonic acid products among others, accumulate within active muscle and stimulate group III and group IV afferent neurons leading to a reflex sympathetic discharge (the Muscle Metaboreflex) (Adreani & Kaufman, 1998; Murphy *et al.*, 2003; Ichinose *et al.*, 2011). The Exercise Pressor Reflex (EPR) is exaggerated in hypertension and related conditions like heart failure, with the over activity of the afferent arms (mechanosensitive and metabosensitive afferents) of this reflex being the cause of the exaggeration (Leal *et al.*, 2008).

Pulse wave amplitude is useful in monitoring sympathetic influences on skin blood flow in the finger and heart rate variability ratio monitors the cardiac autonomic tone (Watanabe *et al.*, 2010; Murphy *et al.*, 2011). Heart rate variability is also an important non invasive predictor of the risks of the metabolic syndrome (Hsiung *et al.*, 2014)

Studies have assessed the modulation of the sympathetic outflow by the muscle metaboreflex (Watanabe *et al.*, 2010; Jarvis *et al.*, 2011). However no study has compared the modulation of the sympathetic nerve activity to the cardiovascular system by the muscle metaboreflex, between individuals with LBW and NBW. In this study it hypothesized that individuals born with LBW would respond with exaggerated

sympathetic discharge to the heart and the vascular system during stimulation of the Muscle Metaboreflex, compared to NBW individuals.

1.5.2 Cardiac fatigue

Research has shown that prolonged strenuous exercise produces a transient decrease in cardiac function (both diastolic and systolic function), a phenomenon referred to as exercise-induced cardiac fatigue. This was demonstrated in both well trained and untrained subjects (Dawson *et al.*, 2003; Oxborough *et al.*, 2010).

Exercised induced studies (Allison *et al.*, 1999)The same method may be useful in determining the risk of developing heart failure in asymptomatic individuals, since there is evidence that prolonged exercise leads to transient reduction in cardiac function by exercise induced cardiac fatigue (Dawson *et al.*, 2003; Oxborough *et al.*, 2010). Low birth weight individuals may be more prone to exercise induced cardiac fatigue because they have a compromised structure and function as highlighted above, making them more susceptible to heart failure.

1.5.3 Exercise induced hypertension

Exercise induced systemic hypertension is used as a predictive tool for future hypertension in asymptomatic normotensive individuals. An exaggerated diastolic blood pressure response during a standard stress exercise protocol test has a strong association, with future onset of resting hypertension (Singh *et al.*, 1999; Allison *et al.*, 1999). Exercise induced hypertension carries a significant risk for major cardiovascular events in healthy asymptomatic normotensive individuals making it a predictive tool for future hypertension. An exaggerated diastolic blood pressure

response especially during the second stage of the standard stress exercise test protocol also has a strong association, about 2- to 4-fold risk for future onset of resting hypertension (Singh *et al.*, 1999; Allison *et al.*, 1999).

1.6 WHY THE SYMPATHETIC NERVE ACTIVITY IN MEDIATING METABOLIC SYNDROME IN LBW

The association between low birth weight (IUGR) and the sympathetic nervous system started being reported more than two decades ago. Shaul *et al* (1989) reported a compromised sympathoadrenal system function in the intrauterine growth restricted foetus or newborn and concluded it may contribute to the pathogenesis of the perinatal morbidities and the increased perinatal mortality known to occur in this high-risk population. Another group showed that babies born after chronic intrauterine stress exhibited a higher adrenergic response with little reserve to counteract stressful situations that made them more vulnerable to disease (Van Reempts *et al.*, 1997). Jansson & Lambert (1999) realising that IUGR was a result of placental insufficiency in the western world rather than maternal nutritional deficiency studied the relationship between birth weight and adult blood pressure and glucose tolerance in an established rat animal model of placental insufficiency. They did not find any relationship between IUGR and blood pressure. However, they found IUGR to be associated with increased sympathetic nerve activity and impaired glucose tolerance in adult life. Since then many others have reported increased sympathetic nerve activity in low birth weight offspring compared to normal birth weight offspring (Zhang *et al.*, 2001; Lumbers *et al.*, 2001; Ward & Phillips, 2001; Young, 2002; Galland *et al.*, 2006; Young, 2006). There are studies however that found decreased sympathetic nerve activity in low birth weight offspring (Weitz *et al.*, 2003) and it is speculated this

maybe a result of whether the offspring had ‘catch-up growth’ or not (Hausberg *et al.*, 2004).

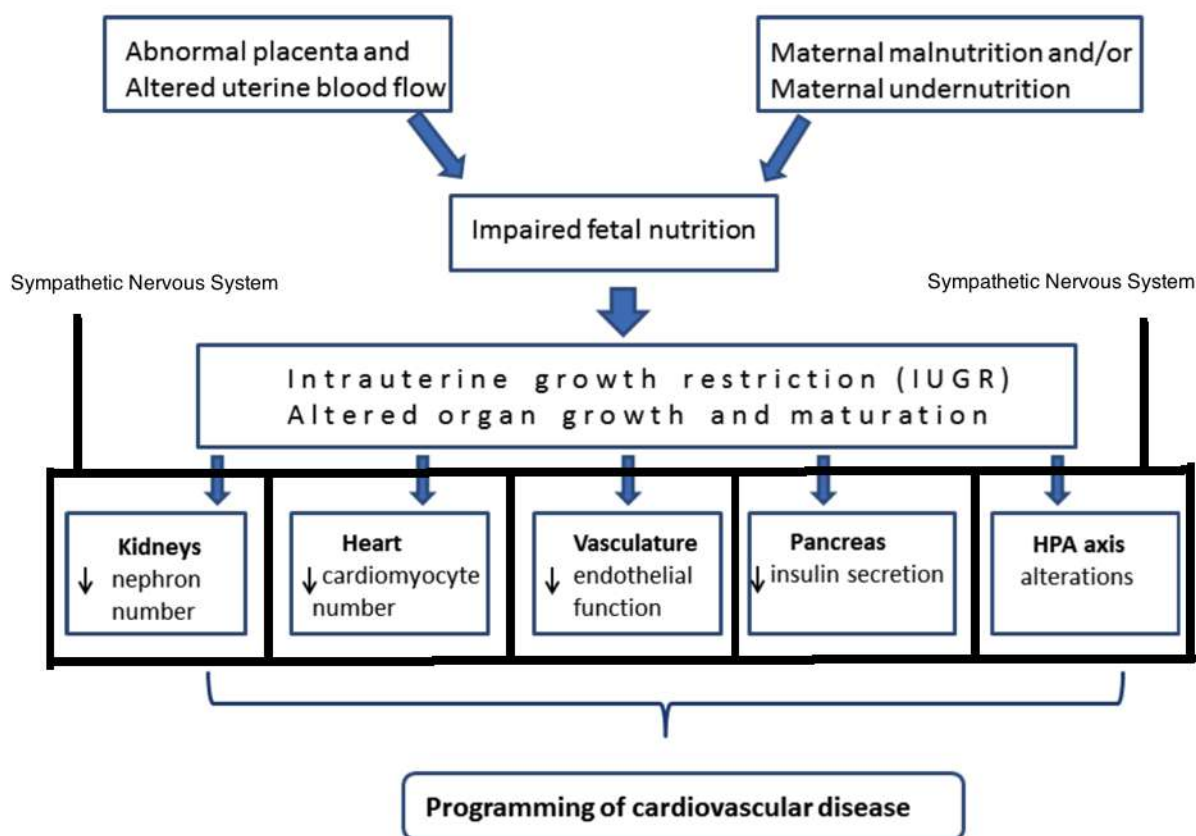


Figure 2: Diagram showing how impaired maternal nutrition and/or abnormal placental function leads to intrauterine growth restriction (IUGR) and subsequent changes to organs (anatomical and physiological) that play a key role in cardiovascular function. These changes have the potential to programme for long-term cardiovascular disease. Modified from Zohdi *et al.* (2014).

Increased sympathetic nerve activity is more likely to be the reason that links LBW or IUGR and cardiovascular diseases in adulthood (Hausberg *et al.*, 2004). Increased sympathetic activity increases vasoconstriction (Holwerda *et al.*, 2014; Roy & Secomb,

2014) which increases total peripheral resistance (Rook *et al.*, 2014; Zicha *et al.*, 2014) leading to hypertension (Gallo *et al.*, 2012; Intapad *et al.*, 2013; Dodson *et al.*, 2013). Stimulation of the sympathetic nerves innervating the juxtaglomerular apparatus results in sodium retention through stimulation of the renin angiotensin aldosterone system (Chifamba 2000; Kopp 2014; Johnson *et al.* 2014). The sympathetic nervous system also elevates blood pressure by vascular structure alteration (Chiu *et al.*, 2012; Voltarelli *et al.*, 2014; Puzdrova *et al.*, 2014). Eutropic remodelling in arteries reduces the sizes of the lumen increasing the total peripheral resistance again elevating blood pressure (Intengan & Schiffrin, 2001).

Despite numerous studies in both animals and humans that low birth weight is associated with cardiovascular diseases, insulin resistance and increased morbidity and mortality in adulthood (Borja, 2013; Alexander *et al.*, 2014; Jaddoe *et al.*, 2014; Visentin *et al.*, 2014), the mechanisms involved remain unknown (Heerwagen *et al.*, 2013; Ikezumi *et al.*, 2013; Nemoto *et al.*, 2014). The inconclusive evidence has been mostly attributable to the experimental protocols used in these studies (Hanson & Gluckman, 2014; Zohdi *et al.*, 2014). However since the sympathetic nervous system regulates blood pressure, metabolic rate and has been demonstrated to be related to the insulin resistance and obesity (Vollenweider *et al.*, 1994; Pulzer *et al.*, 2001) all which have been shown to be important in the pathogenesis of hypertension (Mufunda *et al.*, 1994; Fernandez-Twinn & Ozanne, 2006; Setia & Sridhar, 2009) it is the likely candidate for investigations for the mechanism at play.

Phillips & Barker (1997) selected a cohort of 449 adults to investigate if elevated sympathetic nervous system (SNS) activity could be programmed in utero and be one of the mechanisms mediating low birth weight and insulin resistance and raised blood pressure in adulthood. Here, they reported a direct relationship between adult pulse rate and birth weight. They concluded that elevated SNS activity established in utero is one mechanism linking small size at birth with the insulin resistance syndrome in adult life.

In a case control study of 13 healthy low birth (LBW) subjects (< 2500 g at term) aged 20-30 years and 13 normal birth weight (NBW) subjects (NBW; 3200-3700 g), Weitz *et al.* (2003), showed subjects born too small for their gestational age (SGA) had significantly lower SNA under baseline conditions. They further concluded that the sympathetic response to haemodynamic alteration was not affected in LBW subjects, and maximal activation during non-haemodynamic sympatho-excitatory manoeuvres was preserved. In a later study Weitz *et al.* (2013) reported in another case-control study that autonomous nervous function was not generally deteriorated in young adults with LBW and had a significant association with metabolic rate. Thus making the autonomic nervous system a determinant of the body weight gain leading to cardiovascular risk.

A study by Boguszewski *et al.* (2004) found results contrary to those reported above by Weitz *et al.* (2003). In a cross-sectional, comparative study of 20 healthy adults (21-25 years old) born SGA was associated with increased sympathetic nerve traffic. It

was their conclusion that the increase in sympathetic nerve traffic in young adults born SGA with normal and short stature may be the link between low birth size, hypertension and cardiovascular morbidity later in life inspired this study.

Interest has since been generated proposing that the sympathetic nerve activity was the link between low birth weight and adult hypertension (Hausberg *et al.*, 2004). Both increased and decreased sympathetic nerve activity in individuals born with low birth weight have been reported. The system plays an important role in the regulation of both metabolic and cardiovascular activities. The hypothesis for this study was that the sympathetic nerve activity (over activity and under activity) is the link between low birth weight offspring and later hypertension seen in these offspring when they become adults.

1.7 RESEARCH QUESTION

Research question: Is change in the sympathetic nerve activity the mechanism by which low birth weight offspring are programmed for hypertension in adulthood?

1.8 Objectives of the study

1. Develop a rat model for low birth weight studies
2. Compare the change in diastolic function, E/A ratio, in response to prolonged exercise in low birth weight and normal birth weight individuals

3. Compare blood pressure changes during exercise between low and normal birth weight young Black adults (age ≤ 18 or ≥ 26 years)
4. Compare electrolyte excretion before and after renal denervation in LBW and NBW rat offspring
5. Determine blood glucose levels by conducting an oral glucose tolerance test in low and normal birth weight young black adults.

CHAPTER 2

METHODOLOGY

A cartoon (Figure 3) online on Electrocardiogram Basics by Mohamoud (2014) influenced the methodological approach in this study. Observing something from more than one angle will give a better understanding of the object. In this study, the role of the sympathetic nervous system in the development of hypertension and metabolic syndrome in low birth weight offspring was investigated. Animals (rats), humans (young adults), growth restricted rats, exercising young adults were studied.

Two models of low birth weight or intrauterine growth restriction were used, i.e. one rat

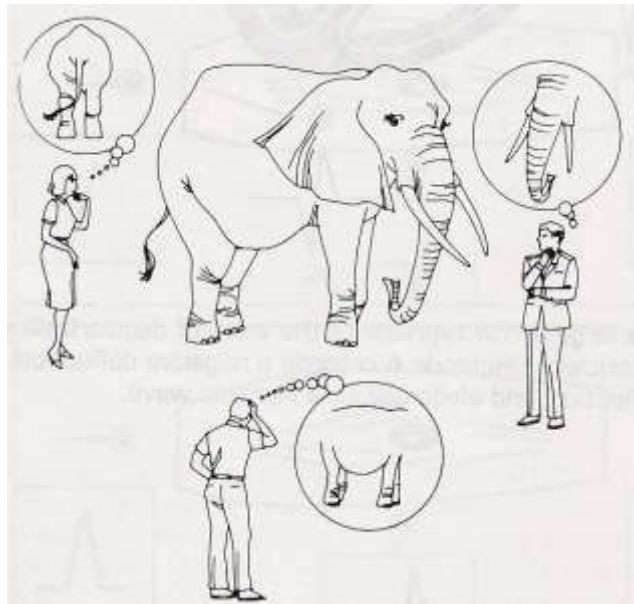


Figure 3: Observing the same object from more than one point you will recognize the object.

animal model obtained by protein restriction and a human model of young adults whose birth weight was below 2.5kg.

2.1 ANIMAL MODEL (SPRAGUE-DAWLEY RAT)

Normal (not genetically modified) Sprague-Dawley rats were purchased from South Africa and kept at the University of Zimbabwe Animal House. The rats were kept in breeding cages under standard conditions of temperature, 22–24 °C with a 12 h light/12 h dark cycle. For breeding purposes, five female rats were caged together with one male rat. Presence of a mucus plug was used as confirmation that the rats had mated successfully and pregnancy expected. Pregnant dams were separated into two groups. One group was fed with standard rat chow (18% protein) (National Food Stock feeds PVT, LTD, Zimbabwe Company) and the other group was fed with a dried and ground *Zea mays* (commonly called locally mealie meal) (also from National Foods) which contains 9% protein as reported in a previous study (Merchant *et al.*, 2005). Mealie Meal was mixed with water and the paste made into balls of 100 g and dried at 60 °C for 24 h before feeding the rats *ad libitum*.

Initially the pregnant dams were placed in cages in groups, but when some of the dams on low protein ate their offspring each pregnant dam was placed in a separate cages. This was a method previously described by Langley-Evans *et al.* in 1994 of inducing low birth weight (intrauterine growth restriction) in rats. Stated briefly, it consisted of feeding dams with low-protein diet for the duration of the pregnancy starting from gestational day one when pregnancy had been confirmed. At birth the pups were nursed by their mothers (whose diet was changed to normal rat chow) until weaned at four weeks of age to standard rat chow. Unlike synthetic diets used by the Langley-Evans *et al.*

(1994), constituents shown in Table 4, mealie meal constituents reported by Merchant *et al.* (2005) and National Foods rat pellets were used. The mealie meal was mixed with water until formation of a paste, which was molded and dried into pellets similar to rat chow pellets.

After birth the pups were separated into two groups, i.e. offspring of mealie meal fed dams (intrauterine growth restricted/low birth weight) and normal rat chow fed dams (non intrauterine growth restricted rats/ normal birth weight).

Table 4: Composition of synthetic normal (18%)-protein (A) and low (9%)-protein (B) diets (Langley-Evans *et al.* (1994))

Constituent	Composition (% by weight)	
	A	B
	18%(w/w) protein	9%(w/w) protein
Casein	18	9
Cornstarch (Snowflake)	42.45	48.45
Cellulose fibre (Solkafloc)	5	5
Sucrose	21.3	24.3
Choline†	0.2	0.2
Mineral mix (AIN-76)*	2	2
Vitamin mix (AIN-76)*	0.5	0.5
D,L-Methionine‡	0.5	0.5
Corn oil‡	10	10
Magnesium sulphate‡	0.05	0.05

Ion	Ionic content (g/kg)	
	A	B
	Na+	0.58
K+	2.15	2.15
Ca ²⁺	2.94	2.94
Mg ²⁺	0.29	0.29
Cl-	0.90	0.90

2.1.2 Rat renal nerve denervation studies

Only male offspring rats were used in the renal denervation and blood pressure measurement experiments because of the blood pressure and sympathetic nerve activity differences between the sexes (Elmes *et al.*, 2009).

2.1.2.1 Surgical preparation

After 10 weeks the rats were weighed and anesthetized using 3-4% halothane and the anaesthetic level was maintained at 1-2% throughout the procedure. The surgical procedures were performed in an antiseptic environment at the University of Zimbabwe veterinary hospital theatre. After shaving the animal in the low abdomen, a midline abdominal incision was made in order to expose the kidneys. Following surgery, the wound was closed and animals allowed to recover in a temperature regulated incubator.

2.1.2.2 Acute renal denervation (ARD)

ARD of both kidneys was carried out by stripping the renal arteries and veins out of their adventitia. All observable renal nerves passing from the celiac and aortic renal ganglia to the kidney were carefully isolated, dissected and cut.

2.2.2.3 Sham operation

The kidneys and renal vessels of the sham operated rats were exposed but their renal nerves left intact.

2.2.2.4 Blood pressure measurement

Mean blood pressure and heart rate were measured using the Biopac (Biopac System, INC CA, USA) for small animal (rat) non invasive blood pressure measure measuring system (Figure 4). Pauline *et al.* (2011) reported on this method and said it was simple, economic, accurate and reliable.

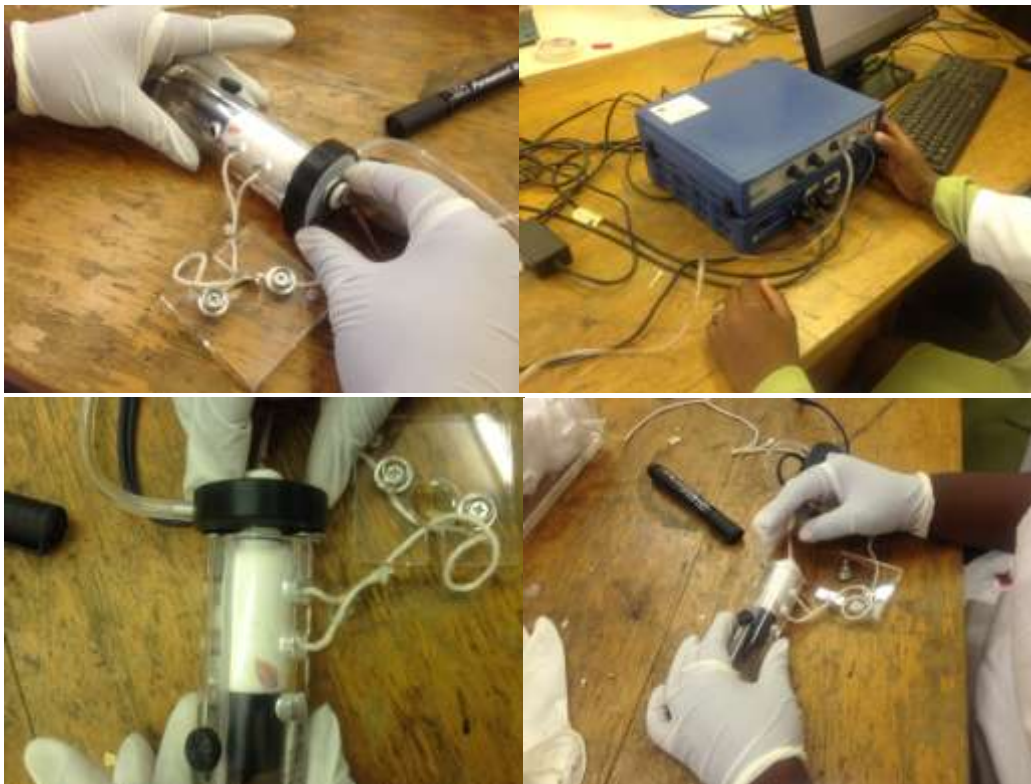


Figure 4: The Biopac (Biopac System, INC CA, USA) for small animal (rat) non invasive blood pressure measure measuring system.

2.2.2.5 Data analysis renal denervation studies

Data is expressed as mean \pm standard error of the mean (SEM) and comparison of values between groups was done using one way and two way analysis of variance

(ANOVA) followed by a Bonferroni/ Dunnet (all mean) post hoc test (Stata version 10.1). The differences between the means were considered significant at a probability value less than 5%.

2.2 HUMAN MODEL

The study participants were selected from pre-clinical medical, nursing science, rehabilitation, physiotherapy, radiography, dental and nutrition undergraduate students at the University of Zimbabwe College of Health Sciences. They were then screened using a self-administered questionnaire. Recruitment was based on documented proof of birth weight on the form of a Ministry of Health, Child Health Card. The exclusion criteria were:

- (i) Age ≤ 18 or ≥ 26 years
- (ii) A history of cardiovascular disease or hypertension (defined as the current use of antihypertensive medication or resting blood pressure $\geq 140/90$ mmHg)
- (iii) Pre-term birth (information is on the birth card)
- (iv) Diabetes mellitus
- (v) Body Mass Index (BMI) greater than 18.5 kg/m^2 less than 24.9 kg/m^2 .

The participants were assigned into two groups, low birth weight (LBW) participants and normal birth weight (NBW) participants. Low birth weight was defined as birth weight $< 2.5 \text{ kg}$ at term.

Joint Parirenyatwa Hospital and College of Health Sciences Research Ethics Committee (JREC) and the Medical Research Council of Zimbabwe (MRCZ) granted ethical permission for the study. Informed consent was sought and obtained from the participants for all protocols.

Sample sizes for the human studies whose protocols are described below were calculated and in most cases all the low birth weight students who volunteered for the study conducted were recruited if they met the criteria. There was no issue with obtaining the normal birth weight students. The studies were conducted on different students in different years. This explains the different sample sizes for the different protocols. An announcement were made in class and notices placed on notice boards about the study. Those willing to participate then gave informed consent and were invited to bring birth cards in the following semester to confirm birth record details.

2.2.1 Metaboreflex studies

The study was conducted in the Exercise Laboratory in the Department of Physiology, University of Zimbabwe. Forty six healthy participants were selected using the criteria already described above including previous endurance training. Before the actual day of study, all participants were provided with the full details of the study and familiarised with the study protocol. In this case control study design, participants were matched for Body Mass Index (BMI).

2.2.1.1 Anthropometric and resting measurements (electrocardiogram and pulse recordings)

Weight and height were measured (scale was SECA model 770) with the participants putting on light clothing (e.g without jackets and jerseys), and without shoes. BMI for each participant was calculated (kg/m^2). Resting blood pressures were measured using mercury sphygmomanometers. Resting Electrocardiogram (ECG) was obtained from lead II and resting pulse wave recordings were obtained from the finger pulse plethysmography for 5-minutes, using iWorx research system hardware (IWX/214) (iWorxSystems, Inc NH 03820 USA) and the Labscribe 2 software. The protocol for measuring Heart rate variability ratio using the above ECG recording and the Labscribe 2 software is described in the iWork website (iWorx, 2013).

2.2.1.2 Exercise protocol (Metaboreflex)

Twenty four hours before exercise, participants were asked to abstain from taking alcohol, energy drinks and caffeinated products. Participants underwent a static/isometric handgrip exercise performed with the dominant hand using a handgrip dynamometer (BIOPAC, Lab BSL 3.7.7, 2008, CA, USA) at 40% maximum voluntary contraction (MVC) until fatigue to ensure that all reached a common metabolic end point. This level of force was chosen since it has been previously shown that handgrips sustained at 40% and 60% of MVC elicited a comparable increase in muscle sympathetic nerve activity (MSNA) (Jarvis *et al.*, 2011). Once the exerted force declined to <80% of the desired force (40% MVC) for >2-seconds, a 2-minute post exercise circulatory arrest (PECA) phase began using an arm cuff inflated to a

pressure >200 mmHg. During the static handgrip exercise and PECA participants were instructed to avoid breath holding. After PECA period, participants were given a 5 min recovery period. Electrocardiograms and pulse wave recordings were obtained during the period of exercise, PECA and recovery.

2.2.2 Cardiac fatigue studies

The study was conducted in the Department of Physiology, University of Zimbabwe, College of Health Sciences, Harare, Zimbabwe. Echocardiography measurements were done by a Sonographer using an Echocardiogram (SonoSite Inc. – US). A total of 23 participants were randomly selected from the subjects described in section 2.2.

Weight and height were measured with the participants putting on light clothing and without shoes using an analogue scale (SECA model 770) on day one of the study protocol. Twenty-four hours before the exercise test, participants were advised to abstain from caffeine and taurine products like coffee and energy drinks that may alter cardiovascular activity during the exercise.

Participants who all were athletes completed a high-intensity (80% VO_{2max}) (Banks *et al.*, 2010; Kane *et al.*, 2011) exercise challenge involving 75 min of running on a treadmill (Trojan Stamina 315 model). All participants completed their exercise in a controlled environment at room temperature. To minimize changes in hydration state and cardiac-loading conditions, participants were encouraged to drink 250 ml of water every 20 min. Post exercise measures were taken within the first 5 minutes after exercise (Banks *et al.*, 2010; Kane *et al.*, 2011).

The following measurements were taken using an echocardiogram by a sonographer who was blinded to the different groups of participants:

- Early mitral inflow velocity (E wave);
- Late mitral inflow velocity using Pulse wave Doppler method.

Diastolic function was determined before and after exercise as the ratio of the early and late mitral inflow velocities (E/A ratio).

2.2.2.1 Data analysis of cardiac fatigue studies

SPSS software version 16.0 (SAS Institute, Cary, NC, USA) was used to analyse the data. Independent samples two-tailed t-test at 5% level of significance was used to compare the various means of metabolic parameters between the two grouping variables, i.e. low birth weight and normal birth weight. Odds ratios were calculated using Pearson chi-square in SPSS at 95% confidence interval. Participants' demographic characteristics and all additional data are reported as means \pm SD.

2.2.3 Exercise induced hypertension

The study was conducted in the Exercise Laboratory in the Department of Physiology University of Zimbabwe College of Health Sciences. Eighty participants were invited to participate from the group described above in section 2.2.

2.2.3.1 *Experimental protocol*

On the day before the exercise test, subjects were instructed to avoid potential stimulants like caffeine and taurine in coffee and energy drinks. Resting blood

pressure was obtained twice from the right arm using a mercury column sphygmomanometer after a 5 minute resting period. The averaged values were used to derive the respective examination resting systolic blood pressure and diastolic blood pressure. During the treadmill tests, the inbuilt Schiller AT60 standard digital sphygmomanometer was used to measure blood pressure and heart rate.

2.2.3.2 Exercise Stress Method

All participants underwent a multistage nine minute exercise ergometer test according to a modified Bruce protocol (Singh *et al.*, 1999). Subjects were to remain on the ergometer for at least two 3 minute stages. Systolic and diastolic blood pressures and heart rate were recorded with the subject on the ergometer immediately before testing and during the last minute of each 3 minute exercise stage.

2.2.3.3 Data Analysis of exercised induced hypertension

SPSS software version 16.0 was used to analyse the data. Independent Samples two-tailed T-test at 5% level of significance was used to compare the various means of cardiovascular parameters between the two grouping variables, i.e. low birth weight (LBW) and normal birth weight (NBW). Chi-square test at 95% CI was done to test for association between LBW and exercise induced hypertension. The data is represented as mean \pm SD on tables and mean \pm SEM on graphs.

2.2.4 Post exercise hypotension

The study was conducted in the Department of Physiology, University of Zimbabwe, College of Health Sciences. From the pool of LBW volunteers, 26 subjects were randomly selected using the selection criteria as already described in section 2.2. A similar criteria was used for to select age matched NBW subject form a pool of NBW volunteers.

2.2.4.1 Experimental Protocol

A method already described in section 2.2.3.1 was used.

2.2.4.2 Exercise stress method

All participants underwent a multistage 12 minute treadmill exercise stress test in accordance to the Bruce protocol (Singh *et al.*, 1999). Two SBP, DBP and heart rate recordings were taken with the subject on the ergometer at 5 minute and 10 minute intervals respectively.

2.2.4.3 Data Analysis post hypertension

SPSS software version 16.0 (SAS Institute, Cary, NC, USA) was used to analyse the data. Independent samples two-tailed t-test at 5% level of significance was used to compare the various means of cardiovascular parameters between the two grouping variables, i.e. LBW and NBW. Subject baseline characteristics are reported as means \pm S.D. and all additional data are reported as means \pm S.E.M.

2.2.5 Glucose tolerance studies

Validation of the glucometers used for this experiment was done at the Department of Chemical Pathology, University of Zimbabwe, Medical School, Avondale, Harare, Zimbabwe. Participants were selected as described in section 2.2. For each LBW participant, a control match for gender, age (± 0.5 yrs), body mass index (± 1)-normal without familial history of diabetes were selected from the larger normal birth weight (NBW) group.

2.2.5.1 Experimental Protocol: Glucose Tolerance Test

All participants had their 12 hour fasting blood glucose levels measured and underwent a 75-gram oral glucose tolerance test for 2 hours according to the World Health Organization standards and American Diabetes Association criteria (Carlsson *et al.*, 1999; American Diabetes Association, 2013).

2.2.5.2 Data Analysis of glucose tolerance studies

SPSS software version 16.0 (SAS Institute, Cary, NC, USA) was used to analyse the data. Independent samples two-tailed t-test at 5% level of significance was used to compare the various means of metabolic parameters between the two grouping variables, i.e. LBW and NBW. Linear regression was done to assess for association between LBW and glucose intolerance in the young black adults. Participants' demographic characteristics and all additional data are reported as means \pm S.E.M. The data is represented on graphs generated using SPSS 16.0 and MsExcel 2010.

2.2.6 Salt retention and hypertension

2.2.6.1 Salt retention protocol

Participants were 66 medical students from the University of Zimbabwe, age 18-26 years selected according to the criteria in section 2.2. The protocol was adapted and modified from a previously published protocol (Nishimuta, 2006) (Figure 5). On the night prior to the experiment, participants avoided dehydrating substances (e.g coffee, drug) and vigorous activities starting at 2000 hours. On the day of the experiment they were given a light breakfast before 0800 hours and refrained from drinking or eating from 0900 hours, 5 hours before commencement of the experiment.

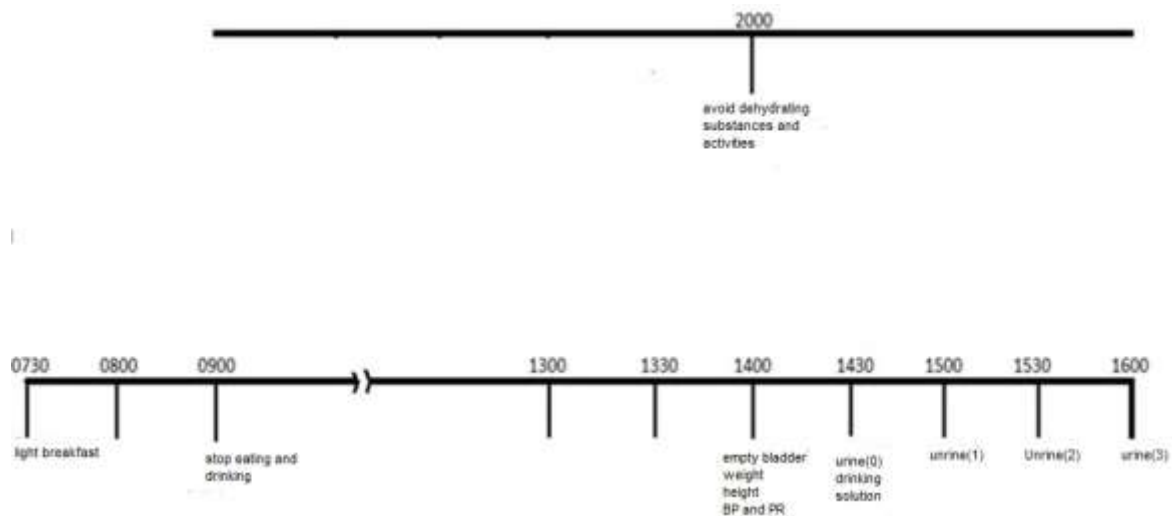


Figure 5: Experimental protocol for salt retention studies adapted from Nishimuta (2006).

At 1400 hours participants emptied their bladders and blood pressure, pulse rate and anthropometrics were measured. Blood pressure was measured using a mercury

sphygmomanometer. Three blood pressure measurements were done on the right forearm in the resting sitting position and the average of the last two readings was taken as the resting blood pressure (BP). Thirty minutes later baseline urine was collected. Immediately after collection of the baseline urine sample, each participant drank 10ml/kg body weight of 0.9% saline. Subsequently, they emptied their bladders at 3 successive 30 minute intervals. During the experiment, a participant would sit quietly in a room and would only leave the room for collection of a urine sample.

Urine volume was measured using a measuring cylinder. Urine Na⁺ and K⁺ were measured using ion selective electrolyte electrode (Beckman Synchrons CX5 Delta Clinical Systems).

2.2.6.2 Data analysis of salt retention studies

Data is recorded as mean (SD) except in graphs where standard error mean was used. Statistical analysis was carried out using SPSS (version 16). Statistics used were dependent and independent t-test and/ or ANOVA. A p value of <0.05 was considered statistically significant.

CHAPTER 3

RESULTS

3.1 ANIMAL MODEL (SPRAGUE-DAWLEY RAT STUDIES)

3.1.1 Intrauterine growth restricted model

A total of 92 pups were born (n=38 for the intrauterine growth restricted rats and n=54 non intrauterine growth restricted rats). In Table 5 the IUGR rats had significantly lower birth weight but in 10 days and weeks postnatal there was no significant difference showing catch up growth in IUGR rats. However during the 10 weeks period there was significant different in blood pressure between the groups.

Table 5: Mean birth weight, weight on day 10 and mean arterial pressure at 10 weeks for intrauterine growth restricted (IUGR) and non intrauterine growth restricted rat (Non IUGR)

	IUGR	Non IUGR	p value
Number	38	54	
Mean Birth weight± SEM (grams)	4.33 ±0.11	5.69 ±0.08	0.0001*
Weight Post natal day 10± SEM (grams)	14.96±1.25	14.59±1.34	0.600
Weight at 10 weeks ± SEM (grams)	165±39	170.89 ± 16.7	0.070
Mean blood pressure at 10 weeks ± SEM (mmHg)	122.46 ± 2.73	106.50 ± 5.81	0.0001*

SEM =Standard Error of the mean * p value significant (p<0.05)

3.1.2 Rat renal nerve denervation studies

Table 6: Mean arterial blood pressure comparison between intrauterine growth restricted (IUGR) (n=10) and non intrauterine growth restricted (non IUGR) (n=10) after surgery in sham and denervated rats

		Non IUGR		IUGR	
		Sham operated	Denervated	Sham operated	Denervated
		99.4 ±11.8 mmHg	98.5±7.9 mmHg	110.62±6.0 mmHg	103.4±8.2 mmHg
Non IUGR	Sham operated 99.36 ±11.81 mmHg		P=1.00		
	Denervated 98.49±7.85 mmHg				
IUGR	Sham operated 110.62±6.03mmHg	p=0.001	P=0.0001		P=0.0001
	Denervated 103.44±8.17mmHg	P=0.066	P=0.013		

P values in Table 6 show that there was significant difference between Non IUGR denervated and IUGR denervated, Non IUGR denervated and IUGR sham operated, Non IUGR sham operated and IUGR sham operated, IUGR denervated and IUGR sham operated blood pressures.

3.2 HUMAN STUDIES

3.2.1 Metaboreflex

A total of 23 LBW (18 females, 5 males) and 23 NBW (14 females and 9 males) black adults, with a median age of 20 years (18-25 years), successfully completed the experiment. The baseline characteristics and the mean maximum voluntary contraction, time to fatigue as well as the heart rate changes are shown in Table 7 and Table 8 respectively.

Table 7: Baseline demographic characteristics of the study sample. Data presented as mean \pm SD.

	Total (n=46) mean \pm SD	NBW (n=23) mean \pm SD	LBW (n=23) mean \pm SD	p value
Age/years	20.24 \pm 0.95	20.13 \pm 1.01	20.35 \pm 0.89	0.443
Body Mass Index (kg/m ²)	20.88 \pm 2.0	20.38 \pm 2.22	21.88 \pm 1.65	0.091
Mean Systolic BP (mmHg)	113 \pm 11.66	112 \pm 11.4	115 \pm 12	0.403
Mean Diastolic BP (mmHg)	73 \pm 9.27	71 \pm 8.5	74 \pm 10	0.213
Mean Heart Rates (beats/min)	83 \pm 10.47	82 \pm 11.3	84 \pm 9.7	0.532
Birth Weight (grams)	2.744 \pm 0.89	3.248 \pm 0.43	2.241 \pm 0.19	0.0001*

NBW= Normal Birth Weight; LBW= Low Birth Weight; * indicates that birth weight was the only baseline variable where there was a significant difference between the two groups.

Table 8: Mean Heart rates during entire protocol and mean maximum voluntary contraction and time to fatigue

	Total (n=46) mean \pm SD	NBW (n=23) mean \pm SD	LBW (n=23) mean \pm SD	p value
MVC (kg)	46.1 \pm 13.4	45.7 \pm 13.4	46.5 \pm 13.7	0.829
TTF (s)	141.1 \pm 13.9	141.8 \pm 13.8	141.2 \pm 14.3	0.884
HR-B (beats/min)	83 \pm 10.47	82 \pm 11.3	84 \pm 9.7	0.532
HR-E (beats/min)	94.9 \pm 10.9	86.4 \pm 8.7	103.4 \pm 4.1	0.0001*
HR-PECA (beats/min)	89.8 \pm 8.2	88.1 \pm 7.6	91.6 \pm 8.6	0.148
HR-R (beats/min)	84.6 \pm 9.3	83.9 \pm 9.8	85.3 \pm 8.9	0.595

MVC= Maximum Voluntary Contraction, TTF= Time To Fatigue, * indicates a significant difference.

3.2.1.1 Heart rate variability ratio

The trends of mean HRV ratios for LBW and NBW individuals during the experimental protocol are shown in Figure 6. The mean increase in HRV from baseline to exercise was greater for LBW compared to NBW individuals (1.015 \pm 1.034 vs 0.119 \pm 0.789, respectively; p<0.05). However, the mean increase in HRV ratio from baseline to

PECA and from baseline to recovery was not significantly different between LBW and NBW individuals (Table 9).

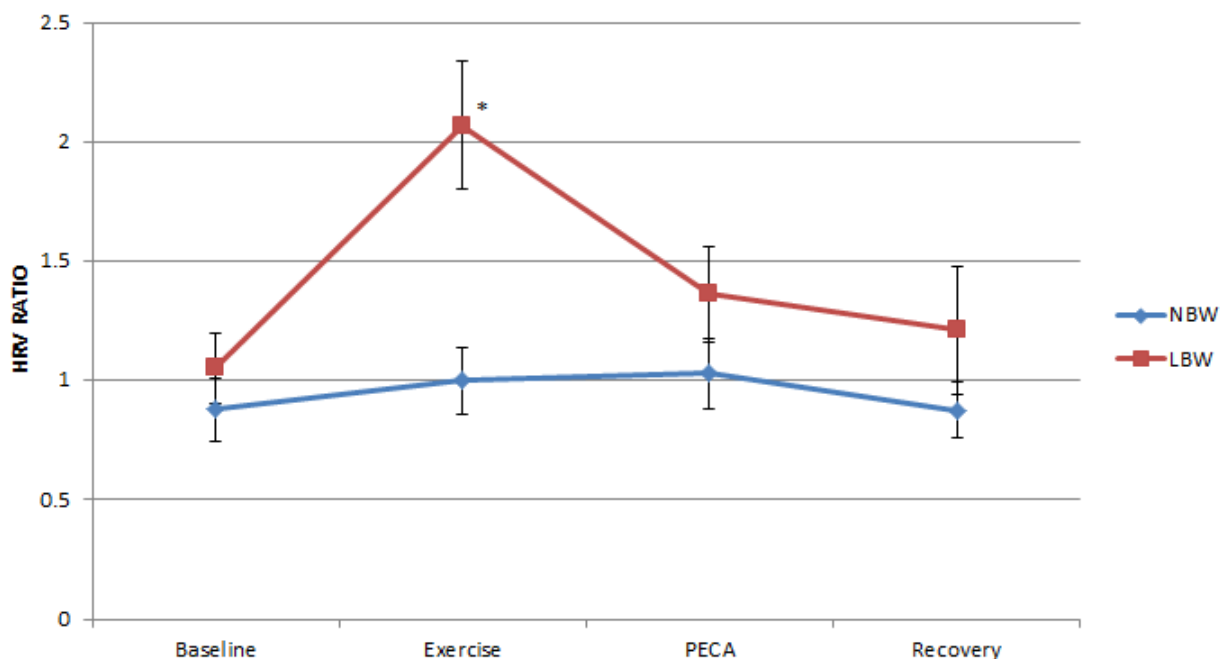


Figure 6: Mean Heart Rate Variability ratio (HRVr) responses during rest, static/isometric handgrip exercise, Post Exercise Circulatory Arrest (PECA) and recovery period, of NBW and LBW individuals. * $p < 0.05$

3.2.1.2 Pulse wave amplitude

The trends of the mean PWA for LBW and NBW individuals during the experimental protocol are shown in Figure 7. The mean decrease in PWA from baseline to exercise was greater for LBW compared to NBW individuals (-1.32 ± 1.064 vs -0.735 ± 0.63 , respectively; $p < 0.05$). The mean decrease in PWA from baseline to PECA was also greater for LBW compared to NBW individuals (-0.932 ± 0.998 vs -0.389 ± 0.563

respectively; $p < 0.05$). However, the mean change in PWA from baseline to recovery was not significantly different between LBW and NBW individuals (Table 9).

Table 9: Mean changes in HRV ratio & PWA from Baseline to Exercise, PECA, or Recovery.

	LBW	NBW	p value
Mean change in HRV ratio from B-E \pm SD	1.015 \pm 1.034	0.119 \pm 0.789	0.002*
Mean change in HRV ratio from B-PECA \pm SD	0.311 \pm 0.679	0.15 \pm 0.71	0.436
Mean change in HRV ratio from B-R \pm SD	0.157 \pm 1.275	-0.003 \pm 0.638	0.593
Mean change in PWA from B-E (mV) \pm SD	-1.320 \pm 1.064	-0.735 \pm 0.63	0.028*
Mean change in PWA from B-PECA (mV) \pm SD	-0.932 \pm 0.998	-0.389 \pm 0.563	0.029*
Mean change in PWA from B-R (mV) \pm SD	-0.421 \pm 1.134	-0.284 \pm 0.635	0.615

B-E=mean change in PWA/HRV ratio from Baseline to Exercise; *B-PECA* =mean change in PWA/HRV ratio from Baseline to PECA and; *B-R*=mean change in PWA/HRV ratio from Baseline to Recovery and. * $p < 0.05$ showing a significant difference between LBW & NBW.

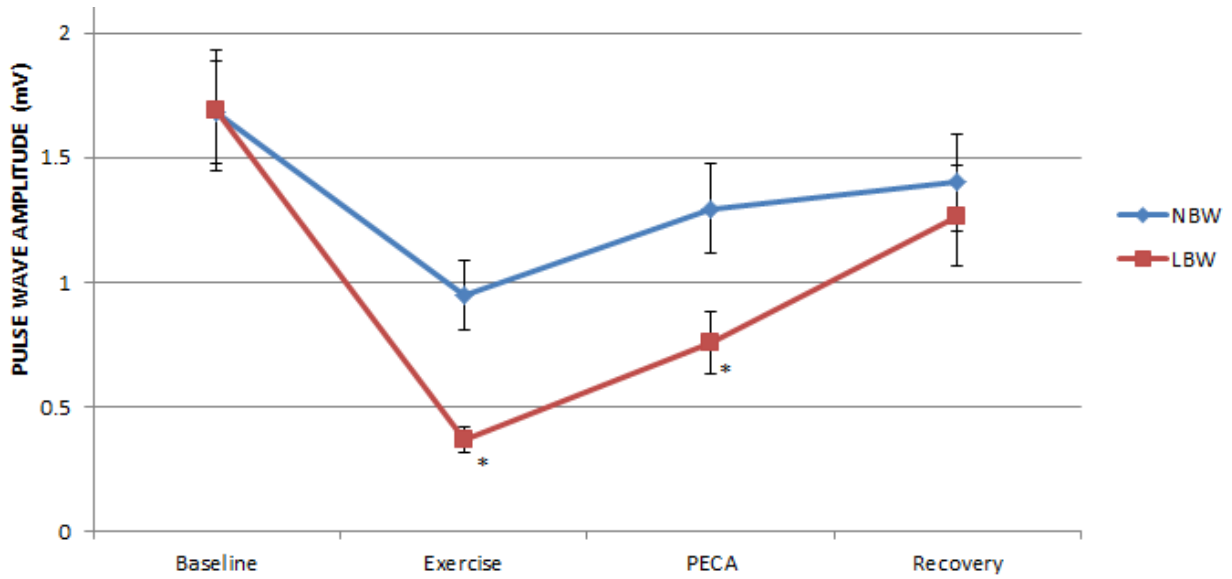


Figure 7: Mean Pulse Wave Amplitude (PWA) during rest, static/isometric handgrip exercise, Post Exercise Circulatory Arrest (PECA) and recovery period, in NBW and LBW individuals. * $p < 0.05$ NBW vs LBW

3.2.2 Cardiac fatigue

3.2.2.1 Demographic Data

A total of 23 young adult participants, nine (39%) females and 14 (61%) males with mean age 20.7 ± 3.3 years were recruited. Seven had low birth weight (3 females and 4 males) and 16 had normal birth weight (6 females and 10 males). Table 10 shows the demographic characteristics of the study sample.

Exercise induced cardiac fatigue was defined as $\Delta E/A$ ratio ≥ 0.40 (Dawson *et al.*, 2003). Out of the 23 participants who were studied, only seven developed EICF (5

LBW and 2 NBW individuals). There was a significant association between low birth weight and EICF (Chi-square test $p < 0.05$, odds risk ratio 4.64; 95% CI 1.19-18.1).

Table 10: Demographics of study sample. LBW = low birth weight; NBW =

normal birth weight, SD=standard deviation. Formulae, $VO_{2max} = 14.8$

$- (1.379 \times T) + (0.451 \times T^2) - (0.012 \times T^3)$ in males and $VO_{2max} = 4.38$

$\times T - 3.9$ in females. * $p < 0.05$; significant difference between NBW and

LBW individuals.

	LBW (n=7)	NBW (n=16)	p value
Age/years	21.2±4.7	20.2±1.6	0.335
BSA/m ²	1.62±0.17	1.70±0.18	0.285
Mean DBP(mm/Hg)	73±11	75±8	0.529
Mean SBP(mm/Hg)	112±16	116±12	0.341
Mean HR(beats/min)	79±10	76±6	0.310
Birth weight/grams	2260±241	3320±505	0.001*
VO _{2max} (ml/kg/min)	59.04±15	61±12	0.224

BSA is Body Surface Area.

Table 11: Haemodynamic measurements, Δ E/A ratio- change in the E/A ratio from rest to post exercise.

	LBW (n=7)	NBW (n=16)	p value
Resting E/A ratio	1.77 \pm 0.15	1.65 \pm 0.33	0.097
Post exercise E/A ratio	1.25 \pm 0.22	1.55 \pm 0.34	0.043*
Δ E/A ratio*	0.48 \pm 0.27	0.19 \pm 0.18	0.031*

*=p<0.05; significant difference between NBW and LBW individuals

3.2.3 Exercise induced hypertension, exercise studies

A total of 80 young black adults, with mean age of 20.4 \pm 1.7 years completed the study. Forty eight females and 32 males participated in the study. Thirty four participants had LBW (26 females and 8 males) and 46 had NBW. The baseline characteristics of the groups are shown in Table 12.

Table 12: Baseline demographic characteristics of the study sample.

	LBW (n=34)	NBW (n=46)	Total (n=80)	p value
Age/ years	20.2 \pm 1.7	20.5 \pm 1.8	20.4 \pm 1.7	0.397
Body mass index(kg/m ²)	20.4 \pm 2.1	20.5 \pm 2.9	20.5 \pm 2.6	0.794
Mean Systolic BP(mm/Hg)	114 \pm 17	112 \pm 14	113 \pm 15	0.695
Mean Diastolic BP(mm/Hg)	79 \pm 11	77 \pm 11	78 \pm 11	0.601
Mean Heart rate (beats/min)	81 \pm 19	80 \pm 16	80 \pm 17	0.876
Birth Weight (grams)	2083 \pm 404	3156 \pm 356	2699 \pm 652	0.000*

Mean \pm SD

3.2.3.1 Exercise Induced Hypertension (EIH)

Exercise induced systemic hypertension was defined as a peak systolic blood pressure of > 200mmHg (Singh *et al.*, 1999). In this study, 32 subjects (40%), nine males and 23 females developed exercise induced hypertension. The proportion of LBW individuals, 62% (n=21), who developed exercise-induced hypertension (EIH) was significantly higher, $p < 0.05$, than the proportion of those with NBW, 32% (n=11), as shown in Figure 8. χ^2 -test carried out showed a significant association between LBW and EIH ($p < 0.001$), odds risk ratio 7.5 (95% CI 2.7-20.7) .

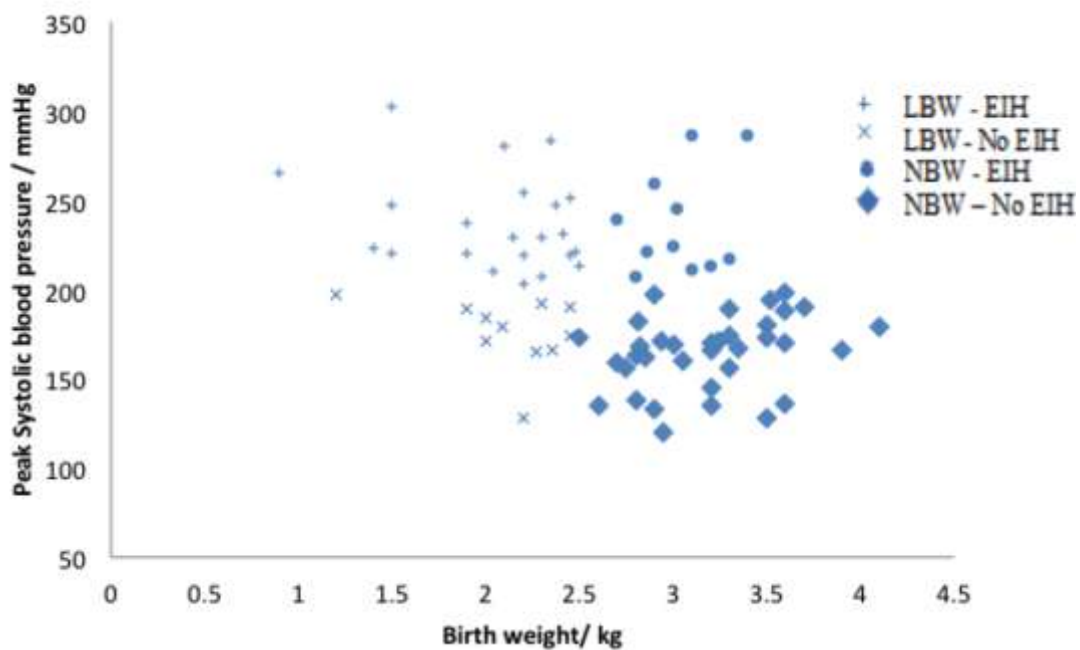


Figure 8: Scatter plot of peak SBP against birth weight. LBW-EIH = low birth weight participants who developed exercise induced hypertension, LBW-No EIH = low birth weight participants who did not develop exercise induced hypertension, NBW-EIH =

normal birth weight participants who developed exercise induced hypertension, NBW-No EIH = normal birth weight participants who did not develop exercise induced hypertension.

3.2.3.2 Systolic blood pressure (EIH)

The mean resting systolic blood pressure (SBP) for the whole group was 113 ± 15 mmHg and there was no significant difference between the LBW and NBW groups, $p > 0.05$ (Table 12).

After Stage I, there was a significance difference in SBP between NBW (155 ± 40 mmHg) versus LBW (190 ± 44 mmHg), ($p < 0.0001$) and the mean SBP remained significantly different on Stage II ($p < 0.0001$). However, in Stage III there was no significant difference between the mean SBP in the two groups. Thus the exaggerated systolic response of the LBW group was only appreciable in Stage I and Stage II of the exercise protocol, as shown in Figure 9.

Table 13: Mean Heart Rate responses during the exercise protocol.

	NBW(n=46)	LBW(n=34)	p value
Resting HR beats/min	80 ± 16	81 ± 19	0.876
HR Stage I beats/min	116 ± 25	125 ± 27	0.108
HR Stage II beats/min	139 ± 29	144 ± 28	0.412
HR Stage III beats/min	147 ± 18	146 ± 26	0.878
Mean \pm SD			

3.2.3.3 Diastolic Response (EIH)

Diastolic blood pressure (DBP) was not significantly different at rest between NBW vs LBW individuals although it was higher for Stage 1 ($76\pm 16\text{mmHg}$ vs $92\pm 25\text{mmHg}$, $p<0.05$) and Stage 2 ($77\pm 17\text{mmHg}$ vs $91\pm 28\text{mmHg}$, $p<0.05$) in LBW versus NBW. This sharp rise in diastolic response is a medical phenomenon that is known as an exaggerated diastolic response (Singh *et al.*, 1999). There was no significant difference in DBP between NBW and LBW during stage III, as shown in Figure 10.

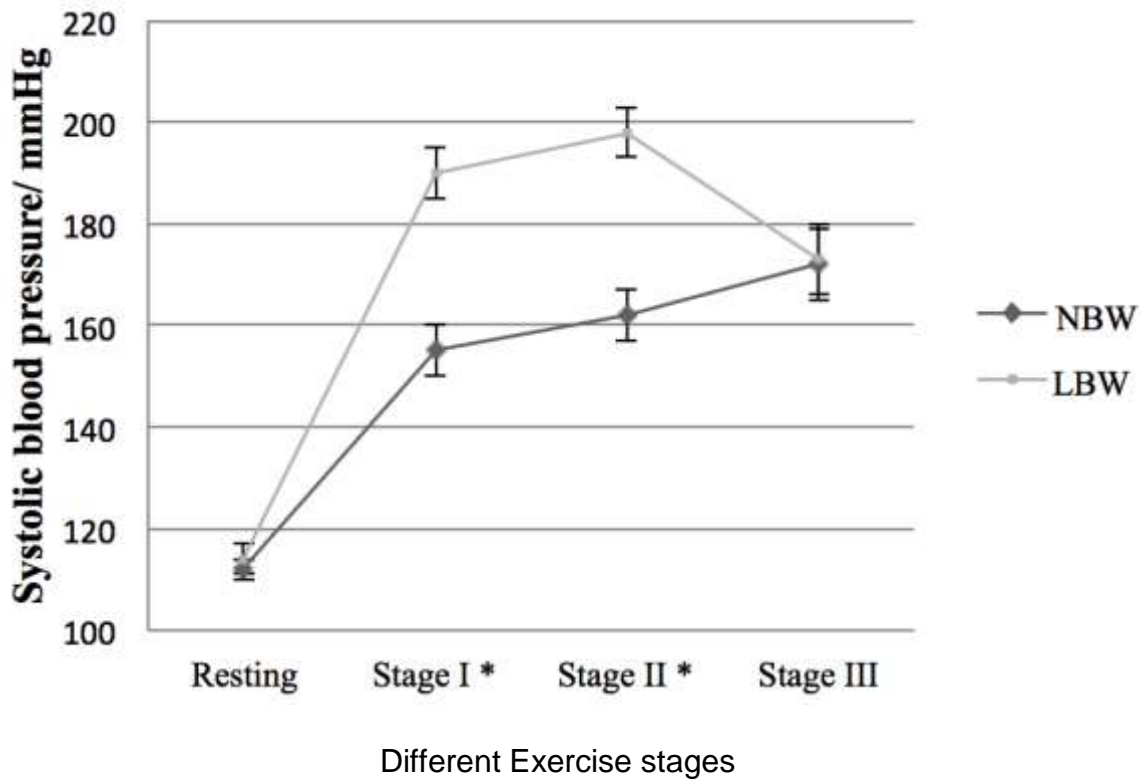


Figure 9: The graph of mean SBP responses during exercise of NBW and LBW

Key: $*=p<0.05$ NBW vs LBW(mean \pm SEM). Stage I = SBP after 3 minutes of exercise, Stage II = SBP after 6 minutes of exercise, Stage III = SBP after 9 minutes of exercise, LBW = low birth weight participants, NBW = normal birth weight participants, $*= p<0.05$ NBW vs LBW.

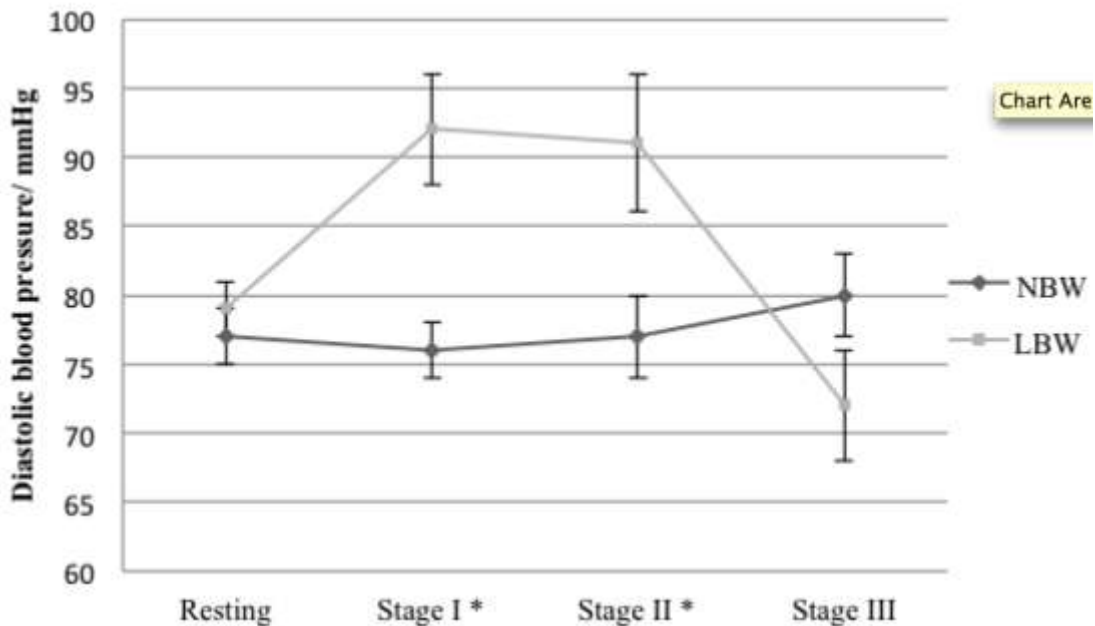


Figure 10: The graph of mean DBP responses during exercise of NBW and LBW $*=p<0.05$ NBW vs LBW (mean \pm SEM). .Stage I = DBP after 3 minutes of exercise, Stage II = DBP after 6 minutes of exercise, Stage III = DBP after 9 minutes of exercise, LBW = low birth weight participants, NBW = normal birth weight participants, $*= p<0.05$ NBW vs LBW.

3.2.3.4 Heart Rate (EIH)

There was no significant difference in the mean resting heart rate (HR) for NBW vs. LBW individuals. There was no significant difference in the mean exercise heart rates for NBW vs. LBW in Stage I, Stage II and Stage III , as shown in Table 13.

3.2.4 Post exercise hypotension (PEH)

A total of 52 young adult subjects, 28 females and 24 males who were of mean age 20.2 ± 1 years were recruited using the above named criteria. Twenty six had low birth

weight (LBW) and 26 had normal birth weight (NBW). The baseline characteristics are shown in table 14.

Table 14: Baseline Demographic Characteristics of the study sample.

	NBM (n=26)	LBW (n=26)	Total (n=52)	p value
Age (yrs)	20.2±1.1	20.1±0.9	20.2±1.0	0.604
Birth weight(g)	3159±333	2226±235	2692±551	0.001*
Weight at 1 year (g)	9026±679	8624±439	8837±501	0.032*
Body mass index kg/m ²	21.2±3.1	20.1±2.3	20.7±2.7	0.151
Mean SBP (mmHg)	122±10	122±11	122±11	0.827
Mean DBP (mmHg)	78±8	78±12	78±10	0.765
Mean Heart rate (beats/min)	82±14	82±18	82±16	0.967

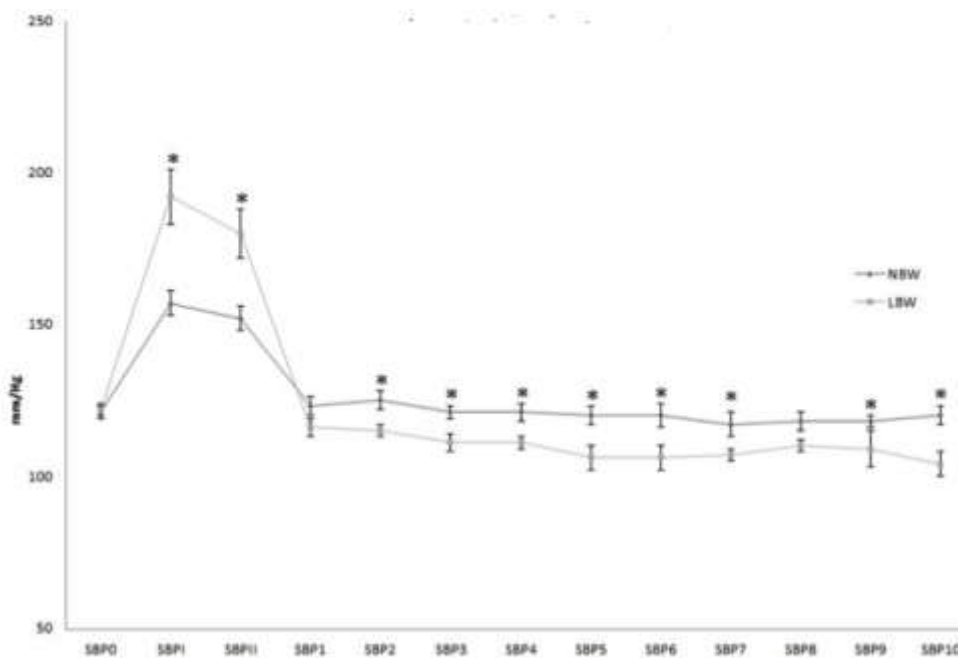
Key: DBP = diastolic blood pressure; LBW = low birth weight; NBW = normal birth rate; SBP = systolic blood pressure; * indicates that birth weight and weight at one year were the only baseline variable where there was significant difference between the two groups.

3.2.4.1 Systolic Blood Pressure (PEH)

There was no significant difference, $p > 0.05$ in the mean resting systolic blood pressure (SBP) of NBW vs LBW group. After 5 minutes and 10 minutes of exercise; SBPI and SBPII on Figure 11 respectively, there was a significant difference ($p < 0.05$)

in SBP between the NBW and LBW groups, with the LBW group having higher exaggerated responses, as shown in Figure 11.

After resting, the SBPs, SBP1 - SBP10, measured every 5 mins were significantly different ($p < 0.05$) between the NBW and LBW groups at all intervals except at 5 minutes and 40 minutes, see Figure 10. The SBP of the NBW returned to baseline levels while the LBW group had a sustained and significantly depressed ($p < 0.05$) post exercise systolic blood pressure measurements which undershoot the resting systolic blood pressure with a mean percentage change of -10.1% (versus NBW's -1.5%), as shown in Figure 10. This sustained undershoot of SBP after a bout of exercise is known as post-exercise hypotension (MacDonald, 2002).



Systolic blood pressure at different stages of exercise

Figure 9: Mean SBP responses during rest, different stages of exercise and post exercise of NBW and LBW individuals. Data presented as mean – standard error of the mean.

Key: LBW = low birth weight participants; NBW = normal birth weight participants; SBP = systolic blood pressure; SBP0 = Resting SBP; SBP1 = SBP after 5 minutes of exercise; SBP11 = SBP after 10 minutes of exercise; SBP1 = SBP 5 minutes post exercise; SBP2 = SBP 10 minutes post exercise; SBP3 = SBP 15 minutes post exercise; SBP4 = SBP 20 minutes post exercise; SBP5 = SBP 25 minutes post exercise; SBP6 = SBP 30 minutes post exercise; SBP7 = SBP 35 minutes post exercise; SBP8 = SBP 40 minutes post exercise; SBP9 = SBP 45 minutes post exercise; SBP10 = SBP 50 minutes post exercise; * $p < 0.05$ NBW vs LBW.

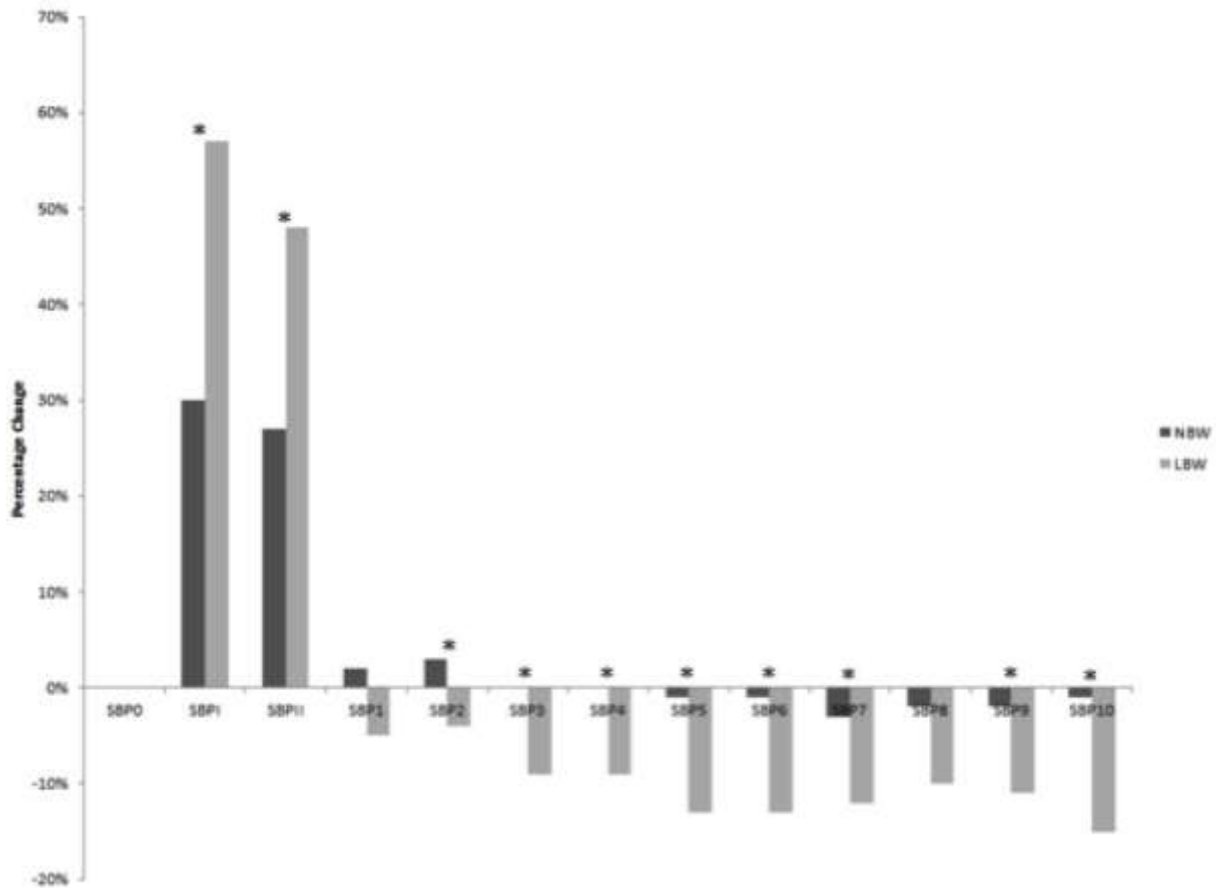


Figure 10: Mean percentage change in SBP responses during rest, different stages of exercise and post exercise of NBW and LBW individuals. Data presented as mean – standard error of the mean.

Key: LBW = low birth weight participants; NBW = normal birth weight participants; SBP = systolic blood pressure; SBP0 = Resting SBP; SBPI = SBP after 5 minutes of exercise; SBPII = SBP after 10 minutes of exercise; SBP1 = SBP 5 minutes post exercise; SBP2 = SBP 10 minutes post exercise; SBP3 = SBP 15 minutes post exercise; SBP4 = SBP 20 minutes post exercise; SBP5 = SBP 25 minutes post exercise; SBP6 = SBP 30 minutes post exercise; SBP7 = SBP 35 minutes post exercise; SBP8 = SBP 40 minutes post exercise; SBP9 = SBP 45 minutes post exercise; SBP10 = SBP 50 minutes post exercise; * $p < 0.05$ NBW vs LBW.

3.2.4.2 Diastolic Blood Pressure (PEH)

The mean resting diastolic blood pressure was the same for both groups. There was no significant difference in DBP during exercise; DBPI and DBPII; although the LBW group had higher diastolic pressures as compared to the NBW, as shown in Figure 11.

The mean post exercise diastolic pressures, DBP1-DBP10, for the LBW group were generally lower and undershot the resting DBP as compared to NBW group. The difference was significant, at DBP5 and DBP10, NBW versus LBW, as shown in Figure 11.

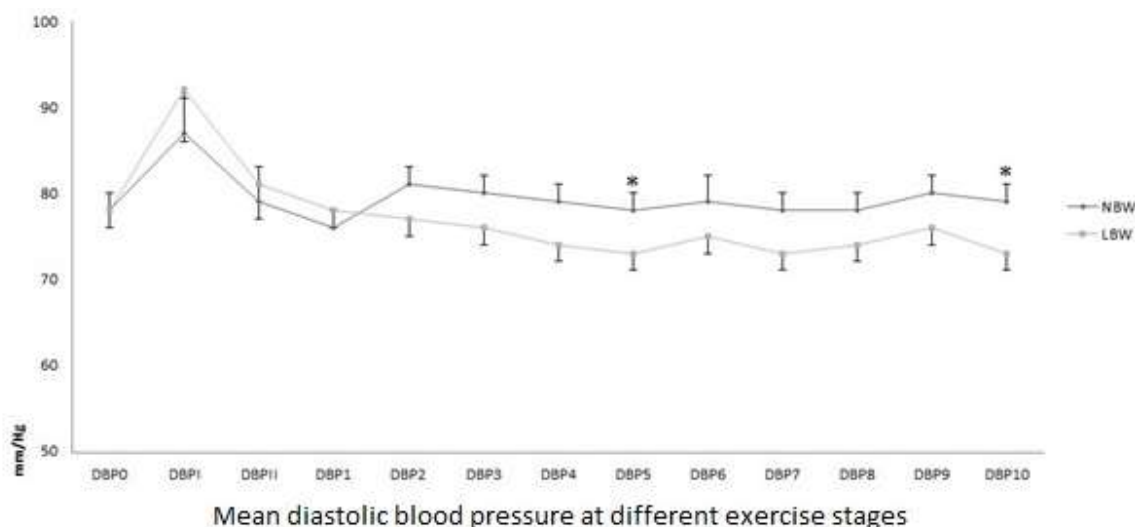


Figure 11: Mean DBP responses during rest, different stages of exercise and post exercise of NBW and LBW individuals. Data presented as mean – standard error of the mean.

Key: LBW = low birth weight participants; NBW = normal birth weight participants; DBP = diastolic blood pressure; DBP0 = Resting DBP; DBP1 = DBP after 5 minutes of exercise; DBP2 = DBP after 10 minutes of exercise; DBP3 = DBP 5 minutes post exercise; DBP4 = DBP 10 minutes post exercise; DBP5 = DBP 15 minutes post exercise; DBP6 = DBP 20 minutes post exercise; DBP7 = DBP 25 minutes post exercise; DBP8 = DBP 30 minutes post exercise; DBP9 = DBP 35 minutes post exercise; DBP10 = DBP 40 minutes post exercise; * p < 0.05 NBW vs LBW.

3.2.4.4 Heart Rate (PEH)

There was no significant difference in the mean resting heart rate for NBW versus LBW individuals. There was no significant difference in the mean exercise HRs for NBW versus LBW, HRI and HRII, as shown in Figure 14.

There was no significant difference in the mean post exercise HRs for NBW versus LBW, HR1- HR10 (Figure 14).Figure 12

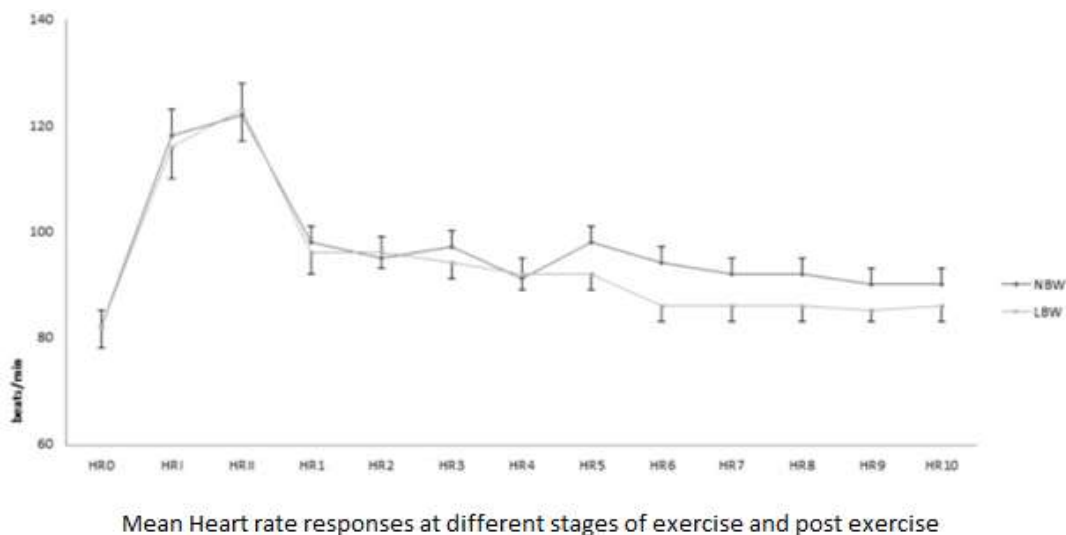


Figure 12: Mean HR responses during rest, different stages of exercise and post exercise of NBW and LBW individuals. Data presented as mean – standard error of the mean.

Key: LBW = low birth weight participants; NBW = normal birth weight participants; HR = heart rate; HR0 = Resting HR; HRI = HR after 5 minutes of exercise; HRII = HR after 10 minutes of exercise; HR1 = HR 5 minutes post exercise; HR2 = HR 10 minutes post exercise; HR3 = HR 15 minutes post exercise; HR4 = HR 20 minutes post exercise; HR5 = HR 25 minutes post exercise; HR6 = HR 30 minutes post exercise; HR7 = HR 35 minutes post exercise; HR8 = HR 40 minutes post exercise; HR9 = HR 45 minutes post exercise; HR10 = HR 50 minutes post exercise; * $p < 0.05$ NBW vs LBW.

3.2.5 Glucose tolerance

A total of 70 young black adults, 47 females and 23 males with mean age 20.28 ± 0.19 years were recruited; 30 had low birth weight (LBW) and 40 had normal birth weight (NBW). Thirty participants out of the 70 had a family history of diabetes (at least one first degree or two second degree relatives with the disease). Table 15 shows the baseline characteristics of the study sample.

3.2.5.1 Oral Glucose Tolerance Test

There was no significant difference, $p > 0.05$ ($p = 0.159$) in the mean fasting blood glucose levels of NBW and LBW groups with baseline values ranging from 4.4 to 5.1 mmol/l (Table 15). After 30 mins and 60 mins of glucose loading; there was a significant difference, $p < 0.05$ in blood glucose levels between the NBW and LBW groups, with the LBW group having higher blood glucose levels, as shown in Figure 13. Ninety minutes and 120 minutes after glucose loading showed no significant difference ($p > 0.05$) in the blood glucose levels between the two groups, although they remained high in the LBW group. Oral glucose tolerance testing detected two cases of type II diabetes (all LBW individuals), 12 cases of impaired glucose tolerance (4 NBW and 8 LBW individuals) and three cases of impaired fasting glucose (all LBW individuals).

3.2.5.2 Regression Analysis of glucose tolerance study

A linear regression analysis was carried out and it showed a strong and negative correlation ($r=-0.277$, $p<0.02$) between glucose levels measured 30 mins after bolus ingestion were used and birth weight; as shown in Figure 14. Increase birth weight was strongly associated with increased regulation of plasma glucose at 30mins.

Table 15: Demographics of the sample in the glucose tolerance study comparing NBW and LBW students .

	Total (n=70) mean \pm SEM	NBW (n=40) mean \pm SEM	LBW (n=30) mean \pm SEM	p value
BMI (kg/m ²)	21.06 \pm 2.0	20.44 \pm 2.31	21.68 \pm 1.55	0.095
Age/years	20.28 \pm 0.19	20.12 \pm 0.22	20.44 \pm 0.16	0.442
Fasting blood glucose levels (mmol/l)	4.79 \pm 0.27	4.47 \pm 0.12	5.11 \pm 0.42	0.159
Glucose levels after 30 mins (mmol/l)	8.33 \pm 0.59	7.24 \pm 0.28	9.41 \pm 0.91	0.029*
Glucose levels after 60 mins (mmol/l)	8.40 \pm 0.56	7.57 \pm 0.36	9.22 \pm 0.75	0.035*
Glucose levels after 90 mins (mmol/l)	7.59 \pm 0.56	6.95 \pm 0.24	8.32 \pm 0.83	0.119
Glucose levels after 120 mins (mmol/l)	6.85 \pm 0.43	6.25 \pm 0.24	7.45 \pm 0.62	0.081
Birth weight (kgs)	2.72 \pm 0.05	3.19 \pm 0.06	2.24 \pm 0.03	0.0001

Key: LBW = low birth weight; NBW = normal birth weight, SEM=standard error mean

* $p < 0.05$; significant difference between NBW and LBW individuals.

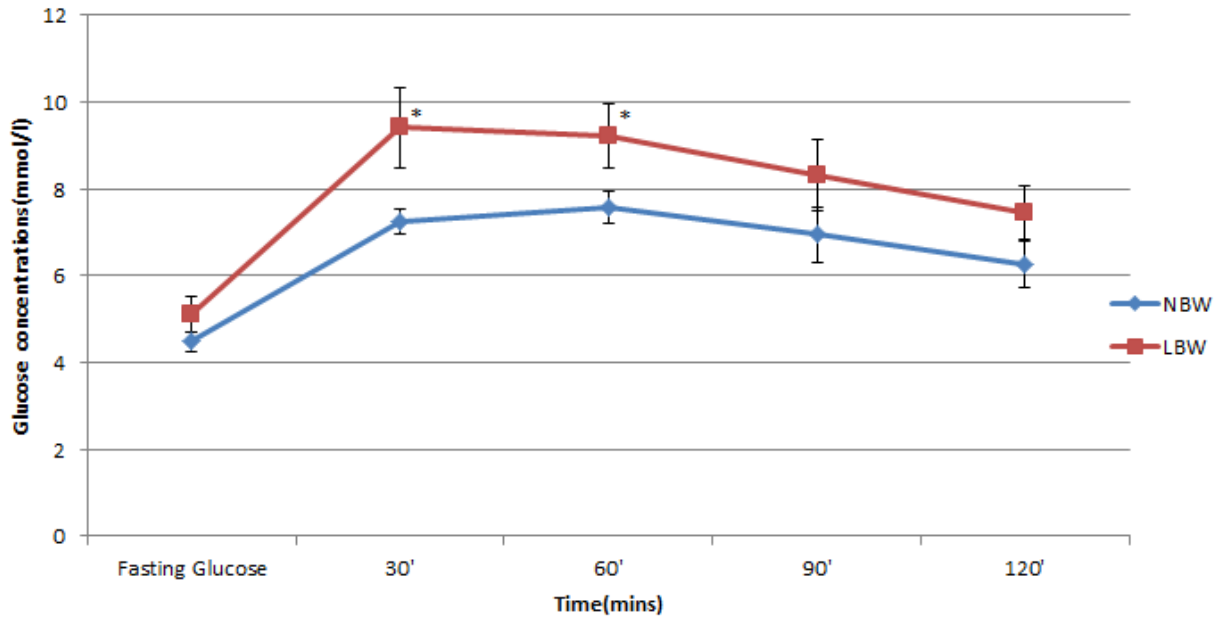


Figure 13: Mean blood glucose levels of fasting blood glucose and the oral glucose tolerance test in NBW and LBW individuals. Data presented as mean – standard error of the mean.

Key: LBW = low birth weight participants; NBW = normal birth weight participants; Following administration of glucose: 30' = blood glucose levels after 30 minutes*; 60' = blood glucose levels after 60 minutes*; 90' = blood glucose levels after 90 minutes; 120' = blood glucose levels after 120 minutes; * p < 0.05 NBW vs. LBW.

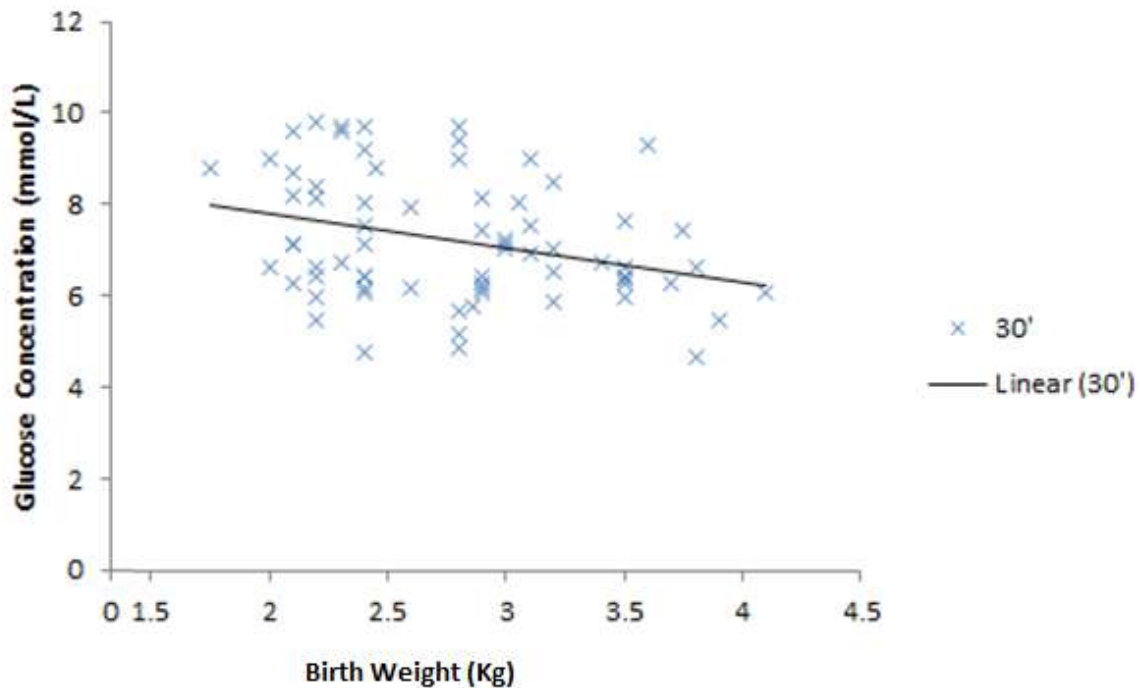


Figure 14: Regression analysis of glucose concentration at 30minutes plotted again birth weight ($r=-0.277$, $p<0.02$).

3.2.6 Salt handling

Of the 66 participants that completed the study, the mean age was 20.5 (1.8) years. Twenty-nine percent ($n=19$) were male and 71% ($n = 47$) were females. Thirty young adults (45%), 8 males and 22 females reported birth weight below 2500g. Table 16 shows age, anthropometric data, resting blood pressure (SBP, DBP) and heart rates (HR) for the NBW and LBW groups. Mean weight for the NBW group, 56.8 ± 9.7 kg was not significantly different from that for LBW, 52.4 ± 11.8 kg ($p=0.105$).

Results for urine Na⁺ and K⁺ excretion and urine flow were compared and they are shown in Figure 17, Figure 18 and Figure 19 respectively. At baseline, NBW group had significantly higher urine Na⁺ excretion than the LBW group (p=0.014). After ingestion of the saline solution urine Na⁺ excretion was higher for the NBW group than the LBW group throughout the experiment but the difference was only significant at 30 minutes (p=0.045). Peak urine Na⁺ excretion in both groups was at 30 minutes. In the NBW, at 90 minutes urine Na⁺ excretion was lower than that at baseline and that in the LBW was still higher than the baseline. The difference between baseline and at 90 minutes for NBW and LBW were not significant (p=0.124 and p=0.173 respectively) (Figure 17).

Baseline urine K⁺ excretion were not significantly different (p=0.369). After ingestion of the saline solution, urine K⁺ excretion increased in both groups with that in the NBW being higher than in the LBW throughout the experiment although the differences were not significant (p>0.05 for all). Peak urine K⁺ excretion in the NBW group was at 30 minutes whereas that in the LBW had a plateau from 30 to 60 minutes (Figure 18).

Baseline urine flow was significantly higher in the NBW than the LBW (p=0.038). After ingestion of saline solution eminent uresis occurred at 30 and 60 minutes being significantly higher in the NBW than the LBW at both instances (p=0.003 and p=0.014 respectively). Peak urine flow was at 60 minutes in both groups (Figure 19).

Table 16: Age and anthropometric data, resting blood pressure and resting pulse rate for NBW and LBW groups.

	Total (n=66) Mean (SD)	NBW (n=36) Mean (SD)	LBW (n=30) Mean (SD)	p value
Age (years)	20.5 (1.8)	20.3 (1.8)	20.7 (1.8)	0.414
Weight (kg)	54.8 (1.1)	56.8 (9.7)	52.4 (11.8)	0.105
Height (cm)	163.9 (8.6)	164.9 (9.3)	162.8 (7.6)	0.342
BMI (wt/ht ²)	20.4 (3.7)	20.9 (2.9)	19.8 (4.4)	0.230
SBP (mmHg)	116 (15)	114 (12)	117 (18)	0.460
DBP (mmHg)	78 (11)	77 (11)	79 (11)	0.486
HR (bpm)	83 (17)	83 (16)	83 (19)	0.851

Key; NBW: normal birth weight; LBW: low birth weight; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; PR: pulse rate.

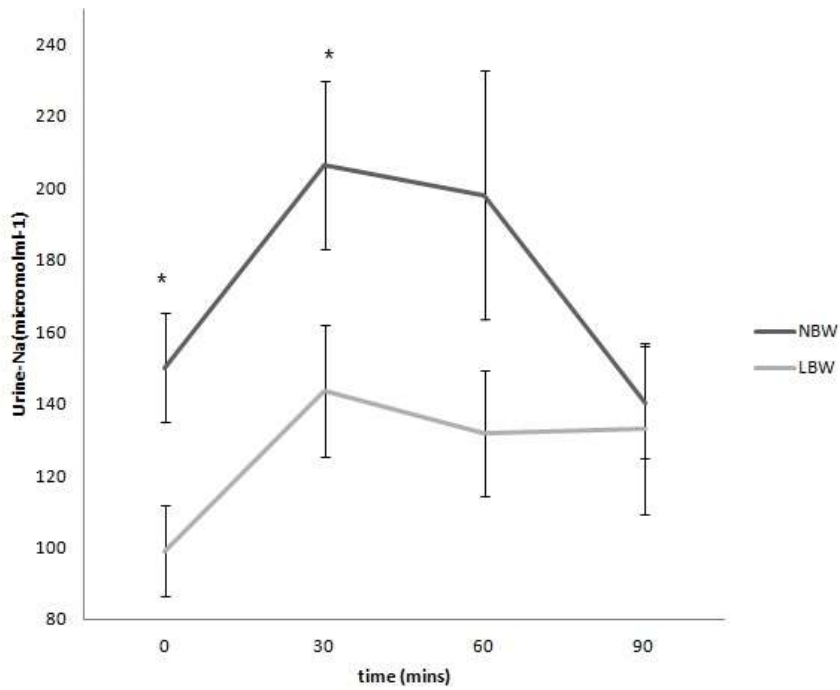


Figure 15: Urine Na⁺ excretion for the normal birth weight (NBW) and low birth weight (LBW) groups. *p<0.05, NBW group compared with LBW group.

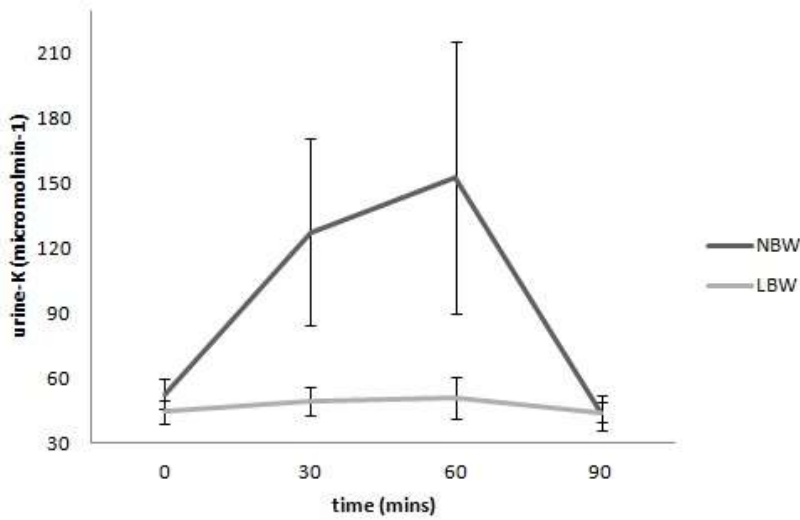


Figure 16: Urine K⁺ excretion for normal birth weight (NBW) and low birth weight (LBW) groups *p<0.05, NBW group compared with LBW.

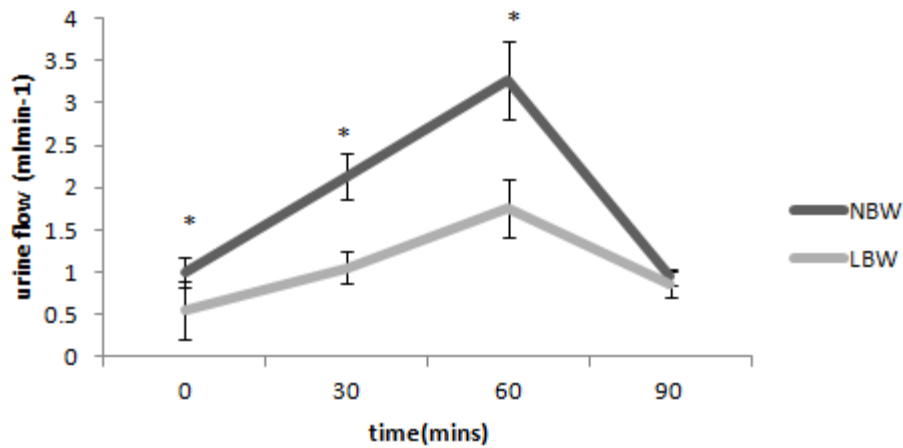


Figure 17: Urine flow for the normal birth weight (NBW) and low birth weight (LBW) groups *p<0.05, NBW group compared with LBW.

CHAPTER 4

DISCUSSION

The sympathetic nervous system has a major role in regulation of the cardiovascular system (Thomas, 2011). This is because it increases the heart rate, cardiac contractility, peripheral resistance by contracting arteries and veins, stimulates secretion of catecholamines and increases fluid retention by stimulating the renin angiotensin aldosterone system. In a series of experiments described in this study it was shown that human offspring born low birth weight and growth-restricted rats have elevated or deranged sympathetic nerve activity. This elevation and deranged sympathetic activity was demonstrated in both LBW rats and young human adults born with birth weight less than 2.5kg. Animal studies allowed the experimenter to give protein restricted diet to rats to obtain low birth weight offspring on which renal denervation studies could be carried out without running into ethical issues that would have occurred in human studies. In Human subject however non-invasive studies could be carried out. Although the rat experiments cannot be extrapolated to human studies at this stage, one extrapolation however exist in clinical practice. Patients with resistance hypertension now have renal denervation done to control their hypertension.

4.1. ANIMAL MODEL

A simple diet of the normal rat chow manufactured by National Foods PVT (Zimbabwean company) and mealie meal (Roller meal manufactured by National

Foods PVT) ubiquitous in Zimbabwe was used in this study. Dams fed on mealie meal, which contained 9% protein, gave birth to pups with significantly low birth weight compared to those on standard rat pellets. A number of low birth weight studies in rats have reported similar values of birth weight values (Payne *et al.*, 2004; Schreuder *et al.*, 2006; Intapad *et al.*, 2013; Boubred *et al.*, 2013). The experimental model of IUGR also demonstrated catch up growth (Schreuder *et al.*, 2006) which has been associated with the development of hypertension and the reason for differences in sympathetic activity in low birth weight offspring (Hausberg *et al.*, 2004). At 10 weeks when blood pressures were compared between the two groups of pups there was no significant difference in weight as a result of catch up growth (Shahkhalili *et al.*, 2010) in pups born of dams fed on 9% protein. This implied that weight was not the reason for the difference in blood pressure. This endorses the notion that it is the birth weight that is the risk factor for the development of hypertension and not the weight of the offspring as a young adult or adult (Chifamba *et al.*, 2012; Chifamba *et al.*, 2014).

A rat model to demonstrate the central role of the sympathetic nervous system in development of hypertension was used. Denervation of the renal nerves significantly lowered blood pressure in intrauterine growth restricted rats to levels found in non intrauterine growth restricted rats, confirming previous studies reported by Ojeda (2013) and Intapad *et al.* (2013).

A simple nutritional dietary protocol has been used to induce intrauterine growth restriction in rats and is comparable to previously reported rat models. Using this model it was demonstrated that cutting renal nerves significantly lowered blood pressure in intrauterine growth restricted rats.

4.2. HUMAN STUDIES

4.2.1. Metaboreflex

HRV ratio and PWA responses to activation of the muscle metaboreflex are exaggerated in LBW compared to NBW individuals. This suggests that the exercise pressor reflex, which is involved in tight regulation of the cardiovascular response to exercise, is persistently dysregulated into early adulthood for LBW individuals (Smith *et al.*, 2006; Murphy *et al.*, 2011) an observation we confirmed in this study.

HRV ratio for LBW was modestly elevated at rest, although there was no significant difference between the HRV ratios of the two groups. This may be due to LBW individuals being prenatally programmed to increase sympathetic nerve activity secondary to the adverse intra-uterine conditions that the fetus will be subjected to (Smith *et al.*, 2006). Studies have shown that the exercise pressor reflex is exaggerated in hypertensive rats, in part due to over activity of the afferents (Taylor *et al.*, 1998; Leal *et al.*, 2008). The efferent component of the exercise pressor reflex was investigated; the afferent pathway has not been investigated in LBW subjects. In hypertensive subjects, exercise evokes an excessive increase in BP from a chronically elevated resting value (Smith *et al.*, 2006). During exercise, compared to baseline, LBW individuals experienced an exaggerated sympathetic response to the static handgrip. This was indicated by a significant mean increase in HRV ratio (increased HRV ratio reflects an increase in sympathetic tone compared to the vagal tone) as well as a significant mean decrease in PWA. LBW and NBW individuals had an increased

sympathetic discharge to the heart and the peripheral vessels, although that of LBWs was exaggerated. The increased sympathetic discharge observed in both groups is in keeping with the findings by Jarvis *et al.* (2011) and Ichinose *et al.* (2011) who described an increase in sympathetic discharge in normal individuals. However, in these two studies birth weight was not considered as a variable while in Watanabe *et al.* (2010) it was. The mean increase in HRV ratio from baseline to PECA decreased but was not significantly different between the two groups, whilst the mean decrease in PWA showed a significant difference between the two groups (LBW and NBW). This indicated a potentiated sympathetic discharge in response to accretion of metabolites in the once active muscle, with the differences in the sympathetic discharge to the heart and the peripheral vessels during PECA being probably due to the fact that there is organ specific sympathetic nerve activity and regional differences in this nerve activity as is experienced particularly in cardiovascular diseases (Schäffer *et al.*, 2008; Jarvis *et al.*, 2011; Chifamba *et al.*, 2012). Animal models in which there was ganglionic and α -adrenergic blockage using hexamethonium and phentolamine respectively, showed an abolishment of the exaggerated pressor response to exercise, suggesting that these exaggerated haemodynamic responses were mediated, in part, by the abnormally large exercise pressor reflex-induced sympathetic nerve activity (Smith *et al.*, 2006). This might be the same reason why the LBW individuals had an exaggerated response to exercise pressor reflex. Exaggerated neuroendocrine and vasoconstrictive responses have been shown to predict the development of hypertension in adults (McCormick Covelli, 2006). Therefore the exaggerated exercise pressor reflex response, in this study, may function as a marker for the pathophysiologic phases of hypertension.

The insignificant difference in mean increase in HRV ratio and significant difference in mean decrease in PWA during PECA may also suggest an increase in cardiac parasympathetic tone buffering the arterial baroreflex (Watanabe *et al.*, 2010). Insensitivity of baroreceptors, which buffer blood pressure increases during exercise, has been reported in hypertensives and individuals with heart failure (Taylor *et al.*, 1998). This might also be present in LBW individuals since they are predisposed to these conditions, therefore future studies to investigate the baroreceptor sensitivity in LBWs are warranted.

Overall, the totality of these findings support the hypothesis that LBW young adults have an exaggerated exercise pressor reflex response that is measurable long before progression to the cardiovascular disorders that LBW individuals are prenatally programmed to develop. The exercise pressor reflex is dysfunctional in cardiovascular disorders such as heart failure and hypertension. This leads to an increased sympathetic discharge underlying the exaggerated increase in BP, heart rate and peripheral resistance during acute physical activity (Taylor *et al.*, 1998; Leal *et al.*, 2008; Murphy *et al.*, 2011). Low birth weight may be associated with an exaggerated vasomotor sympathetic outflow during the muscle metaboreflex activation during exercise in normotensive young adults. LBW individuals may have an exaggerated exercise pressor reflex response.

4.2.2 Cardiac fatigue

This study is the first to elucidate the relationship between birth weight and exercise induced cardiac fatigue with diastolic dysfunction. The findings showed a significant difference between the decrease in diastolic function (E/A ratio) in low birth weight and normal birth weight groups and a significant association between low birth weight and exercise induced cardiac fatigue with diastolic dysfunction. This is in keeping with the hypothesis that low birth weight individuals have structural and functional impairments due to intra-uterine growth retardation, making them more prone to exercise induced cardiac fatigue (Geelhoed & Jaddoe, 2010; Thornburg *et al.*, 2011).

Exercise induced cardiac fatigue study focused primarily on trained athletes participating in prolonged endurance (Dawson *et al.*, 2003; Oxborough *et al.*, 2010). The strenuous exercise and high motivation of athletes due to the competitive nature of these events provides a unique model in which to view cardiac dysfunction. The study protocol involved high intensity (80% VO_{2max}) exercise for 75 minutes as individuals who are not competitively engaged in an endurance exercise programme appear to have a much lower threshold and less tolerance for prolonged endurance exercise. Thus, these non competitive individuals experience cardiac fatigue after a shorter amount of time and at lower exercise intensities than competitive and recreational endurance athletes (Dawson *et al.*, 2003; Oxborough *et al.*, 2010).

Low birth weight individuals showed an even greater sensitivity to prolonged exercise as their diastolic function decreased markedly within 75 minutes. This could be further

explained by epigenetic modification that occurs in response to intrauterine hypoxia and lack of nutrition, leading to a decrease in protection against injury in the left ventricular myocardium (Thornburg *et al.*, 2011), besides structural and functional impairments in utero (Geelhoed & Jaddoe, 2010; Thornburg *et al.*, 2011).

Furthermore, the mechanisms of exercise induced cardiac fatigue involve transient myocardial stunning and a decrease in sensitivity of myocardium to catecholamines reducing the effect of adrenaline in improving both systolic and diastolic function during exercise (Dawson *et al.*, 2003; Oxborough *et al.*, 2010). These factors may be impaired to some extent in low birth weight individuals thus making them more prone to exercise induced cardiac fatigue. In fact, low birth weight is associated with chronic sympatho-excitation later in life as measured by high noradrenaline 'spillover' in low birth weight animals and children (Reynolds *et al.*, 2009; Banks *et al.*, 2011). This also arises from the restricted growth leading to stress induced increases in the circulating levels of cortisol and other glucocorticoids and this prenatal excessive glucocorticoid activity might increase the hypothalamic-pituitary-adrenal (HPA) axis with the resultant high sympatho-excitation (Banks *et al.*, 2011). Hence if there is high sympathetic discharge at rest, β receptor sensitivity to catecholamines, which are already abundant, may easily and quickly decrease during exercise. Low birth weight is associated with exercise induced cardiac fatigue with diastolic dysfunction in young black adults. Hence, low birth weight may be a pathophysiological risk factor for the development of diastolic dysfunction and/or cardiac failure with normal ejection fraction in low birth weight individuals later in life.

4.2.3 Exercise induced Hypertension

Comparison of cardiovascular responses between low birth weight and normal birth weight individuals exposed to an exercise stressor was investigated. It is important to note that hypertension is a multifactorial complex condition with an intertwining of environmental and genetic factors and the influence of prenatal programming is an additional facet to this complex disease (Covelli *et al.*, 2007).

The usefulness of exercise testing lies in the fact that it provides an accurate estimate of blood pressure response to normal physical stress and measurements made during such a test have been shown to be reproducible (Allison *et al.*, 1999).

Some publications have described an association between exercise induced hypertension and the development of future hypertension (Allison *et al.*, 1999; Singh *et al.*, 1999). Indeed Allison *et al.* (1999) reported a relative risk of 2.41 for exercise hypertension in subjects with normal resting blood pressure as compared to those who did not develop exercise hypertension. In a normal individual, exercise is accompanied by a modest rise in systolic blood pressure due to an increase in cardiac output, with no appreciable change in diastolic blood pressure due to peripheral vasodilation. However, this homeostatic mechanism may sometimes be aberrant leading to exaggerated, abnormal blood pressure responses. Although much of the precise neurobiological mechanisms of exercise induced hypertension is still largely unknown, there have been suggestions that for some reasons, a 'hyperactive-

neurogenic' state appears to precede established hypertension in some patients (Allison *et al.*, 1999). This phenomenon is hypothesized to be mediated by two processes; physiological down regulation of the cholinergic sympathetic vasodilator receptors in peripheral tissues due to chronic sympathetic stimulation; and increased wall thickening and consequently the loss of the intrinsic vascular reactivity resulting from exposure to elevated blood pressure (Allison *et al.*, 1999). This correlates well with both human and animal studies by IJzerman *et al.* (2003) and Alexander (2006), who both reported an increase in sympathetic tone in low birth weight infants and mouse pups respectively. Alexander (2006) further demonstrated that renal sympathectomy actually abolished the development of hypertension in low birth weight mice.

In a report by Franz (1987), an exaggerated systolic blood pressure response to exercise was the most consistently used and was likely to be the most reliable and reproducible predictor of future hypertension on the various cardiovascular parameters of an exercise stress test. It is worthwhile to speculate that the mechanisms underlying an exaggerated response might be the same mechanisms underlying the development of exercise induced hypertension and that an exaggerated systolic blood pressure response might be a mild form of the condition. In addition, the increase in in-utero vascular resistance and impaired angiogenesis might be the underlying cause in impaired vascular reactivity resulting in an exaggerated systolic response in low birth weight individuals (IJzerman *et al.*, 2003).

These results are in concordance with a report by Woelk *et al.* (1998) in a retrospective cohort study of a group of 752 Zimbabwean children where they reported a weak inverse association between low birth weight and resting systolic pressure.

Although some studies have observed an association between higher resting systolic blood pressure and the subsequent development of resting hypertension, a number of publications have actually suggested that exercise induced hypertension is a better and more reproducible predictor of future hypertension (Franz, 1987).

The relatively higher resting systolic blood pressure might result from a prenatally programmed increase in sympathetic tone, (Ijzerman *et al.*, 2003) resulting from an adverse intrauterine environment since increased sympathetic discharge results in an increase in cardiac output resulting in a rise in systolic blood pressure.

Normally, moderate exercise is accompanied by a significant increase in systolic blood pressure with no appreciable changes in diastolic pressure due to marked peripheral vasodilation. Such an exaggerated diastolic response as observed in this study might be due to impairment of the normal vasoreactivity probably arising from down regulation in the peripheral sympathetic cholinergic vasodilator receptors (Singh *et al.*, 1999), which can conceivably be attributed to chronic prenatally programmed sympathetic discharge. In this regard, adolescents who had low birth weight were

found to have impaired vascular growth especially in the ascending aorta and such observations might also explain an exaggerated diastolic response (Brodzki *et al.*, 2005).

In a Framingham Heart Study publication, Singh *et al.* (1999) reported that an exaggerated diastolic response during an exercise stress test carried a 2-4 fold risk to development of cardiovascular events, hence is the strongest predictor of future resting hypertension.

In this particular study, a comparison of the mean resting heart rate between normal birth weight group and low birth weight group showed no significant difference. To date, there are conflicting publications regarding an association between low birth weight and heart rate, with some publications describing a weak inverse association (Ijzerman *et al.*, 2003) and some publications describing no association (Woelk *et al.*, 1998).

One can conclude within reason that these data support the premise that low birth weight may predispose to exercise induced hypertension and exaggerated systolic and diastolic responses during exercise in young normotensive adult blacks. These findings may add to support that low birth weight is a pathophysiological risk factor for the development of hypertension as they enter into adulthood and later life. Increased blood pressure in low birth weight young adults and growth-restricted rats was

demonstrated. To reach hypertension levels much longer periods of the investigations were required.

4.2.4. Post exercise hypotension

Hypertension, as described by Hypertension Writing Group (HWG), is a progressive cardiovascular syndrome arising from complex and interrelated etiologies, early markers of the syndrome are often present before precise blood pressure elevation is observed (Giles et al., 2005). The influence of prenatal programming is an interesting additional facet to this complex syndrome (Martyn et al., 1995; Palinski & Napoli, 2008; Kearney et al., 2004).

While post exercise hypotension has been well documented in humans with both borderline hypertension and hypertension (MacDonald, 2002), in normotensive individuals, it is found to be of lesser magnitude than in hypertensive individuals (MacDonald, 2002; Giles et al., 2005). In healthy normotensive individuals, systolic blood pressure is moderately reduced by a mere 5-10 mmHg following a single bout of dynamic exercise in most individuals (MacDonald et al., 1999; MacDonald, 2002; Halliwill, 2001) and this response involves a sustained increase in systemic vascular conductance that is not completely offset by ongoing elevations in cardiac output (Halliwill, 2001). As elaborated and demonstrated by Halliwilli *et al.* (Halliwill et al., 1996; Halliwill, 2001) it appears several mechanisms operate to produce this moderate sustained peripheral vasodilatation following exercise in individuals; (i) the baroreceptor reflex is reset to defend a lower pressure following exercise thus post exercise sympathetic vasoconstrictor outflow is reduced in humans, (ii) and there is

reduced vascular responsiveness to a given level of sympathetic nerve activity (Halliwill et al., 1996; Halliwill, 2001; Lynn et al., 2009).

As highlighted in an earlier publication (Chifamba et al., 2012), sometimes the homeostatic mechanism that finely tune blood pressure might be aberrant leading to abnormal blood pressure responses. Results of this study are in concordance with a number of both human and animal studies where derangements in sympathetic tone were reported in LBW infants (IJzerman et al., 2003; Phillips & Barker, 1997) and pups (Alexander, 2006), respectively. Thus it might be worthwhile to speculate that perhaps the deranged sympathetic tone in LBW individuals might manifest in abnormal cardiovascular hemodynamics which become apparent during exercise and post-exercise thus such exercise induced hypertension and post exercise hypotension might indeed be some of the so called HWG early markers of hypertension. Low birth weight was (is) associated with post exercise hypotension in normotensive young black adults. These findings may aid to support the notion that low birth weight is a pathophysiological risk factor for deranged cardiovascular hemodynamics and the development of hypertension in low birth weight individuals.

4.2.5. Glucose tolerance studies

The oral glucose tolerance test demonstrated that there were significant differences, $p < 0.05$ ($p = 0.029$ for 30 minutes and $p = 0.035$ for 60 minutes) for blood glucose levels between LBW and NBW individuals at 30 and 60 minutes after administration of 75g of glucose. This phenomenon was in line with the evidence that LBW individuals are programmed in utero for future metabolic disorders such as glucose intolerance (IJzerman *et al.*, 2003; Schwartz & Morrison, 2005; Calkins & Devaskar, 2011). After

90mins and 120mins, the oral glucose tolerance test showed no significant difference ($p>0.05$) in the blood glucose levels between the two groups, although they remained high in the LBW group. The 30 and 60 minutes outcomes of the oral glucose tolerance test indicated that LBW individuals were at an increased risk of glucose intolerance and the significantly high blood glucose levels may be as a result of insulin resistance or less insulin secretion. Oral glucose tolerance testing detected 12 cases of impaired glucose tolerance, 4 NBW and 8 LBW individuals. Impaired glucose tolerance was defined according to WHO standards as a postprandial blood glucose level of $>7.8\text{mmol/l}$ up to 11.1mmol/l and diabetes, $>11.1\text{mmol/l}$ (Carlsson *et al.*, 1999). The mean postprandial blood glucose levels for LBW and NBW individuals were $7.45\pm 0.62\text{mmol/l}$ and $6.25\pm 0.24\text{mmol/l}$ respectively. Though there was no significant difference, $p>0.05$ ($p=0.081$), 8 (11%) LBW individuals out of the 70 participants were glucose intolerant. The mean postprandial blood glucose levels in LBW individuals were higher than in NBW individuals and close to 7.8mmol/l which demonstrates that there is a risk of developing glucose intolerance in this group of LBW young adults.

LBW individuals have an increased sympathetic nerve activity and insulin levels may be normal or elevated (American Diabetes Association, 2013). LBW is prevalent in Zimbabwe (UNICEF, 2015) and it might be a significant risk factor for glucose intolerance as indicated by this study.

Previous studies done in Sweden, Iceland, United States and Chinese populations have demonstrated that LBW is associated with glucose intolerance and it is a

significant risk factor for diabetes later in life (Birgisdottir *et al.*, 2002; Saldana *et al.*, 2003; Xiao *et al.*, 2008). Most of these studies were conducted in older adults and few studies in young adults (Levitt *et al.*, 2000; Al Salmi *et al.*, 2008). The onset and extend of metabolic disorders may vary with age. Of all the oral glucose tolerance tests conducted in all these previous studies, this particular study is the first to indicate a significant difference in blood glucose levels 30 and 60 minutes after the test in low and normal birth weight young black adults. Other studies demonstrated that low birth weight was associated with impaired fasting glucose, impaired glucose tolerance and diabetes (Thomas *et al.*, 2006; Eriksson *et al.*, 2006; Coutinho *et al.*, 2013).

The relevance of carrying out a 75g oral glucose tolerance test for 120 minutes is that it is a simple laboratory test and with the American Diabetes Association guidelines; it is a standard test to assess glucose metabolism (Carlsson *et al.*, 1999; IJzerman *et al.*, 2003). This particular study has indicated that low birth weight may predispose to glucose intolerance. Low birth weight was associated with an increased risk of glucose intolerance at 30 and 60 minutes after the oral glucose tolerance test in this group of young black adults. These findings may add to support that low birth weight is a pathophysiological risk factor for glucose intolerance and the development of type II diabetes in low birth weight individuals as they enter into adulthood and later life. Outcomes of this particular study can serve as a baseline for an epidemiological study with a larger sample to statistically determine significant risks and associations of low birth weight and glucose intolerance. Insulin levels should be measured along with conducting an oral glucose tolerance test to explain the changes in blood glucose levels during the test.

4.2.6 Sodium retention studies

The purpose of the study was to investigate renal function, specifically urine Na⁺ excretion in NBW and LBW young adults. The study was conducted under the hypothesis that LBW young adults have a reduced urine excretion of Na⁺ resulting from a congenital nephron deficit and this may play a critical role in the development of hypertension in adult life. The results of the current study add support to the foetal programming hypothesis.

Baseline urine Na⁺ was significantly higher in the NBW than the LBW. This indicates that LBW individuals may have a reduced capacity to excrete urinary Na⁺. In support, after ingestion of the saline solution the lower urine Na⁺ excretion in the LBW compared to the NBW also suggests that LBW young adults may have a reduced capacity to excrete urinary Na⁺. This may be a result of a reduced glomerular filtration surface area caused by a congenital nephron deficit (Brenner et al., 1988; Luyckx & Brenner, 2005). Hughson *et al.* (2003) in an autopsy study of kidneys from 37 African-Americans and 19 Caucasians found that birth weight is a strong determinant of nephron number. They found a linear relationship between birth weight and nephron number. On the other hand Stein *et al.* (2006) after conducting a study of renal function in young adult Caucasians concludes that although the effect of LBW is still small at young adult, IUGR marked by LBW is associated with a low kidney function. Impaired renal handling of Na⁺ observed in the current study shows a low kidney function, which may be a reflection of the congenital nephron deficit. Animal models of

IUGR have shown an under endowment of nephrons in offspring of maternal protein restriction (Augustyniak *et al.*, 2010). Since impaired renal handling of Na^+ is necessary to sustain all forms of hypertension, long term retention of Na^+ by LBW young adults may lead to maintained elevated blood pressure and/ or hypertension. A leading theory supported by Zandi-Nejad *et al.* (2006) is that reduced nephron endowment leads to impaired Na^+ excretion, salt and water retention and may thereby play a causal role in prenatally programming to adult life hypertension.

In addition to a reduction in the glomerular filtration surface area, LBW in animals has been shown to alter expression of transporter proteins in the nephron. Manning *et al.* (2002) reported that maternal protein restriction in rats was associated with lower weight at birth, development of hypertension at 8 weeks and a significant increase in the expression of the bumetanide sensitive co-transporter and thiazide sensitive co-transporter. This may also be the case in humans, however further studies need to be done to ascertain whether it is nephron number or function that is mainly affected by LBW.

Urine K^+ excretion in the NBW and LBW were similar at baseline. The higher increase in the NBW than the LBW that was observed after ingestion of saline was significant. This is supported by results from a study by Vászárhelyi *et al.* (2000) in young Caucasian men, which reports that renal K^+ excretion is not different in the NBW and LBW. Results for K^+ are not clear how this could relate to nephron number and hypertension in adult life. The increase in urine K^+ may be secondary to the increased

delivery of Na^+ and fluid to the distal nephron. Peak urine K^+ excretion occurred at the same time as the peak urine Na^+ excretion.

Urine flow in the NBW was significantly higher than in the LBW at baseline and after ingestion of saline suggesting that LBW individuals retained more fluid. Ideally the glomerula filtration rate calculation was necessary, however its still possible to conclude from the results that LBW individuals retained more fluid and shown by decreased urine output.

The proposed increase in the expression of the Na^+ co-transporters (BSC1) in the thick ascending limb increases the tonicity of the renal medulla. The hypertonic renal medulla absorbs fluid from the collecting duct leading to reduced fluid excretion. Long-term retention of fluid expands the extracellular fluid volume leading to blood volume expansion, volume overload and the subsequent development of hypertension (Brenner et al., 1988; Zandi-Nejad *et al.*, 2006). The relationship between Na^+ renal handling, intravascular fluid homeostasis and hypertension initially described by Guyton *et al.* (1984) is well accepted. Considering the young adult age and the black African race in this study, the study was necessary and it adds support to the foetal programming hypotheses. It is concluded that LBW young adults may have impaired renal handling of Na^+ as indicated by reduced urine Na^+ excretion and they retain more fluid. Collectively these may predispose and serve as early markers for the development of hypertension in adult life.

4.2.7 Integration

The sympathetic nervous system at the juxtaglomerular apparatus stimulates beta receptors to increase renin secretion which stimulates the renin angiotensin aldosterone system resulting in fluid retention and increase total peripheral resistance (Chifamba, 2000). Cutting the renal nerves removed the sympathetics involvement the the intrauterine growth restricted rats and this significantly reduced blood pressure in IUGR rats than non IUGR rats. The LBW young adults with intact renal nerves were shown to significantly retain sodium and as shown in the glucose tolerance studies the elevated sympathetic activity in LBW would result in this fluid retention and over time develop into hypertension or diabetes mellitus. Hausberg et al., (2004) in his review of the possible role of the sympathetic nervous system in the development of the metabolic syndrome noted overactivity was associated with vasoconstriction, in renal retention of sodium and would structural vascular alteration as noted in the diastolic function study. He goes to report however that under activity of the sympathetic nerve activity would affect energy expenditure and result in obesity.

4.3 Conclusion

In the studies reported the dysregulation of sympathetic nervous system is common denominator as in normal physiology shown in Figure 2, it is also the common denominator in LBW and IUGR offspring as the mechanism associated with the development of metabolic syndrome particularly hypertension.

4.4. Study limitations and recommendations

The rat model will be useful in hypertension studies in Zimbabwe, the challenges faced in this study were measuring blood pressure using the cuff method. Investment into telemetry equipment (Malpas, 2010) will provide both accurate and continuous measurement of blood pressure, sympathetic nervous activity and other cardiovascular variables. Blood pressure was measured at certain points in the animals growth and for sympathetic studies nerves had to be cut, however with telemetry these can be measured continuously after insertion of the sensors and without cutting the renal nerves. As mentioned in the introduction, its not possible to acetain the cause of low birth weight in human subjects and at this point difficult to know the impact on the results of this investigation.

CHAPTER 5

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APPENDICES

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