PLACENTAL FUNCTION, BODY COMPOSITION AND CARDIOVASCULAR AUTONOMIC FUNCTION

by

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DECLARATION

This thesis is of my own composition and to the best of my knowledge, contains no material previously published or written by another person, except where due reference is made. Material in this thesis has not been accepted for the award of any other degree at this or any other institution.

Signature:…… Date:

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AUTHORSHIP ATTRIBUTION STATEMENT

This thesis contains one published paper (Chapter four) and two papers currently under review (Chapter three and five). Chapters two, six and seven are currently been prepared for journal submission. Chapters three to seven are part of two cross sectional studies. For all papers and chapters I was involved in all aspects of the work from designing the studies, recruiting participants, collecting data, analyzing data, statistical analysis and interpreting data and finally writing of publications. As a result of my role associated in this research, I am thus the first author in three manuscripts and joint first author in one manuscripts.

Chapter two of this thesis is research review which will be submitted for publication as: **Dissanayake HU**, Skilton MR, Polson JW. *"Autonomic Dysfunction in Programmed Hypertension."*

- For this review paper I conceived the idea, did the appropriate literature searches and wrote all sections of the review paper.
- My co-authors made the following contributions: JWP and MRS supervised my writing as well as edited the manuscript.

Chapter three of this thesis is an original research article, currently under journal review as: **Dissanayake HU**, Anderson L, McMullan RL, Caterson ID, Hyett JA, Phang M, Raynes-Greenow C, Poslon JW, Skilton MR, Gordon A. *"Influence of maternal and placental factors on newborn body composition".*

- For this paper, I made the following contributions: help design the study, recruit participants along with collection of medical records data and placentas. Grading and

staging maternal and fetal inflammatory response using placental reports, statistical analysis and drafting the manuscript.

- My co-authors made the following contributions: LA analyzed majority of the placentas providing a placental report and provided key input to the design of the study. AG provided key input into the design of the study as well as interpretation of the results. RLM helped recruit participants, assisted in interpretation of placental reports and supervised collection of medical records and questioners. MRS provided key input into statistical analysis and along with all other co-authors provided important input into the final manuscript.

Chapter four of this thesis is an original research article published as: **Dissanayake HU**, McMullan RL, Gordon A, Caterson ID, Celermajer DS, Phang M, Raynes-Greenow C, Skilton MR, Polson JW. Noninvasive assessment of autonomic function in human neonates born at the extremes of fetal growth spectrum. Physiological reports. 2018 Apr;6(8):e13682-.

- For this paper, I made the following contributions: help design the study, recruit participants along with recording of all vascular autonomic indices in newborns, statistical analysis of all vascular autonomic indices and wrote all sections of the manuscript.
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- My co-authors made the following contributions: RLM is co-first author on this publication with me. RLM did all cardiac structure and function and aortic intima-media thickness measurements, help recruit participants and oversaw the collection of all medical records and questionnaires. YK analyzed all the aortic intima-media thickness measurements (data not included in this thesis). MRS provided key input into the design of the study as well as statistical help and writing of the manuscript. JWP provided key input into the collection and analysis of autonomic indices and along with all other co-authors provided important input into the final manuscript.

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ABSTRACT

Hypertension is an important modifiable risk factor for cardiovascular and cerebrovascular diseases. Its high prevalence combined with the morbidity and mortality associated with secondary complications make it a major public health concern.

One of the important recent advances in hypertension research is an understanding that hypertension often may have a developmental origin. Birthweight is associated with hypertension across the lifespan and adult cardiovascular disease, such that those at both ends of the spectrum are at increased risk. Nonetheless, birthweight is a relatively crude surrogate of fetal growth and it may be that quantification of body composition, such as percentage body fat, may more accurately identify the "at risk" individual. A causative mechanism linking birthweight and risk of cardiovascular disease is yet to be identified but may involve changes to the structure and function of organs including the placenta which may impair development and predispose individuals to later cardiovascular disease.

Aims

The aims of this thesis were to investigate the associations between placental function, body composition and cardiovascular autonomic function. To achieve these aims, the following associations were tested in three settings:

1. the relationship between maternal factors, placental factors, inflammation and infant body composition
2. the associations of infant body composition and late preterm birth with cardiovascular autonomic function

3. the association of body composition with autonomic functions in the child and adolescent

Results

The study detailed in **chapter three** is a cross sectional study that investigated the relationship between maternal factors, placental function and perinatal inflammatory exposure on the outcome infant fat mass in a cohort of 136 full term infants. This study found that infant fat mass was associated maternal factors (pre-pregnancy weight, maternal age and parity) and placental factors (weight and surface area). A mediation model was employed to determine whether placental function (placental weight) may potentially mediate the association of maternal factors, specifically; pre-pregnancy weight and age. Our mediation analysis revealed selected maternal factors associated with infant fat mass differed from those that were associated with placental weight. Such that only 25-35% of these effects of maternal pre-pregnancy weight and maternal age on newborn fat mass was mediated by placental weight.

The studies detailed in **chapters four and five** is a cross sectional study that investigated the effect of infant body composition on cardiovascular autonomic function. **Chapter four** details whether vascular autonomic function, specifically blood pressure variability and baroreflex function, differ by newborn percentage body fat and prematurity in a cohort of 70 infants, of which 8 were late preterm. Furthermore, this study has introduced a possible new non-invasive measure of autonomic regulation of the myocardium, the dP/dt_{max} variability of arterial pressure. This study found little evidence for altered autonomic function of vasomotor function and cardiac contractility in these groups, with the exception of infant with high body fat who showed reduced baroreflex sensitivity. Furthermore, across the entire body fat spectrum (n=62), there was a nonlinear association between newborn body fat and

spontaneous baroreflex sensitivity that was independent of birthweight. **Chapter five** details the effect of infant body fat percent on cardiac autonomic control and whether body fat percent is a better predictor of alterations in cardiac autonomic control in a cohort of 132 infants, 37-42 week's gestation. Cardiac autonomic control was assessed using heart rate variability (time and frequency anlaysis) in 4-minute epochs repeated up to three times. This study demonstrated that infants with low and high body fat percent have reduced cardiac autonomic control. The non-linear association of body fatness with cardiac autonomic control was independent of birthweight.

The studies detailed in **chapter six** is a cross sectional study that describe cardiac autonomic function in those born late preterm. This study included 26 late preterm newborns at 34 and 37 completed week's gestation and a control group of 114 newborns at 37 and 42 week's gestation. Cardiac autonomic control was assessed using the same methodology as described in chapter five. This study demonstrated that infants born late preterm have reduced overall cardiac autonomic control, reduced parasympathetic modulation of the heart and increased sympathetic modulation to the heart.

Finally, the study detailed in **chapter seven** investigated the association between body composition and autonomic function in children and adolescents 2 to 20 years of age (n=72) at rest and in response to a stress test. Body composition was assessed by air displacement plethysomography using the BOD POD. Markers of autonomic function included; heart rate variability (HRV), systolic blood pressure variability (SBPV), baroreflex function and dp/dt variability measured for 5 minutes during rest and 4 minutes during moderate exercise on a cycle ergometer. This study demonstrated that at rest the association between body fat percent and high frequency SBPV was significantly modified by age. Subjects who were leaner showed increased HRV, increased cardiac parasympathetic drive and lower heart rates, this association was strongest among younger participants, 2-9 years.

In response to exercise, individuals with higher body fat percent showed increased HRV whereas leaner subjects showed lower HRV. The change in HRV from resting to exercise was only associated with fat free mass, such that those who were lean showed a reduced response in HRV to exercise.

Conclusions

These studies indicate different mechanism control fat mass and fat free mass in the newborn and that placental weight partly mediates the association of maternal factors with newborn body composition. The role of the placenta and factors which affect birthweight have gained increasing attention due to the large body of evidence linking birthweight and later disease. While low birthweight has previously been shown to be associated altered autonomic function in the infant our studies suggests that body fatness may provide information beyond that obtained from birthweight assessment alone.

Previous studies have shown altered blood pressure control in those born preterm, our studies found altered cardiovascular outcomes even in born late preterm. Assessment of body composition in children and adolescents at rest and in response to an exercise test suggests worsening of autonomic control due to adiposity may develop over time during childhood and adolescence.

Collectively, these results emphasise the implications of altered in-utero and early life exposures on cardiovascular outcomes.

ABBREVIATIONS

ANS	Autonomic nervous system
BP	Blood pressure
BPM	Beats per minute
BPV	Blood pressure variability
BEI	Baroreflex effective index
CO	Cardiac output
DBP	Diastolic blood pressure
ECG	Electrocardiography
HF	High frequency
HR	Heart rate
HRV	Heart rate variability
IUGR	Intrauterine growth restriction
LF	Low frequency
LGA	Large for gestational age
min	Minutes
mmHg	Millimeter of mercury
MAP	Mean arterial pressure
n	Number of replicates
p	Probability
PI	Pulse interval
PP	Pulse pressure
PNS	Parasympathetic nerve activity
PVN	Paraventricular nucleus
RMSSD	Square root of the mean squared differences of successive NN intervals

SBP	Systolic blood pressure
sBRS	Spontaneous baroreflex sensitivity
SDNN	Standard deviation of the NN interval
SDΔNN	Standard deviation of the difference between adjacent NN intervals
SGA	Small for gestational age
SNA	Sympathetic nerve activity
SV	Stroke volume
TPR	Total peripheral resistance
VLF	Very low frequency

PUBLICATIONS

1. **Dissanayake, H. U**., McMullan, R. L., Gordon, A., Caterson, I. D., Celermajer, D. S., Phang, M., ... & Polson, J. W. (2018). Noninvasive assessment of autonomic function in human neonates born at the extremes of fetal growth spectrum. *Physiological reports*, *6*(8), e13682.

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CONFERENCE PRESENTATIONS

International - oral presentations

1. Newborn Body Fatness and Autonomic Function: Identification of Infants at Risk of Later Cardiovascular Disease. **Hasthi Dissanayake**, Rowena McMullan, Melinda Phang, Kirsty Mckenzie, Yang Kong, Adrienne Gordon, Jon Hyett, Camille Raynes-Greenow, David Celermajer, Jaimie Polson, Michael Skilton. Experimenal Biology, Chicago 2017.

National - oral presentations

1. Associations of placental inflammation, structure and function with infant body fatness and maternal body mass index. **H Dissanayake**, L Anderson, R McMullan, M Phang, M Skilton, A Gordon. Early Mid-Career Research Symposium, Sydney, 2017.

2. Newborn body composition and markers of autonomic function: a potential tool for identification of newborns at risk of later cardiovascular disease. **Hasthi Dissanayake**, Rowena McMullan, MelindaPhang, Kirsty Mckenzie, Yang Kong, Adrienne Gordon, Jon Hyett, Camille Raynes-Greenow, David Celermajer, Jaimie Polson, Michael Skilton. Lifespan network, Sydney, 2016.

3. Newborn body fatness and non-invasive assessment of autonomic function: a potential tool for early identification of cardiovascular risk? **Hasthi Dissanayake**, J.W. Polson, R. McMullan, K. Mckenzie, Y. Kong, A. Gordon, J. Hyett, C. Raynes-Greenow, N. Evans, D.

Celermajer, M. Skilton. Central cardiovascular & Respiratory Control Conference in Sydney, October 2015.

National – poster presentations

1. Maternal saturated fat intake during pregnancy and offspring autonomic function. **Hasthi Dissanayake**, Kirsty Mckenzie, Rowena McMullan, Melinda Phang, Yang Kong, Adrienne Gordon, Jon Hyett, David Celermajer, Jaimie Polson, Michael Skilton. Developmental Origins of Health and Disease, June, 2016

2. An early childhood intervention to improve cardiovascular outcomes. Phang, M, McMullan R, Meroni, A, **Dissanayake, H**, Early-Mid Career Research Symposium, September 2016.

3. Non-invasive assessment of autonomic function in term newborns: a potential tool for early
identification of cardiovascular risk? **Hasthi Dissanayake**, Rowena McMullan, Melinda Phang, Kirsty Mckenzie, Yang Kong, Adrienne Gordon, Jon Hyett, David Celermajer, Jaimie Polson, Michael Skilton. Developmental Origins of Health and Disease, June 2016.

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6. Finalist in the Lifespan Collaboration Award, 2016 – The University of Sydney.
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CHAPTER ONE

INTRODUCTION

HYPERTENSION

Hypertension is defined as a systolic blood pressure ≥ 140 mmHg and a diastolic blood pressure ≥ 90 mmHg (1). At its early stages, hypertension rarely causes symptoms and therefore many people go undiagnosed. Hypertension is an important modifiable risk factor for cardiovascular morbidity and mortality, affecting approximately 40% of people. Its high prevalence, combined with the morbidity and mortality associated with secondary complications, make it a major public health concern. Hypertension is an independent risk factor for heart failure, myocardial infarction, kidney disease, hemorrhagic and ischemic stroke and premature death. If left untreated, hypertension is associated with the continuous increases in cardiovascular risk as well as the onset of vascular and renal damage (2).

According to recent estimates by the World Health Organization, cardiovascular disease accounts for approximately 17 million deaths a year, accounting for one third of the total deaths globally. Of these, hypertension accounted for 9.4 million, being responsible for 45% of deaths due to heart disease and 51% of deaths due to stroke (1).

The increasing prevalence of hypertension is attributed to a combination of factors including population growth, ageing and a complex interaction between behavioral risk factors including unhealthy eating, physical inactivity, weight gain and exposure to persistent stress (2). Over the years, the number of people with hypertension rose from 600 million in 1980 to 1 billion in the year 2008 (1). Currently, successful treatment of hypertension remains poor, and it may be partly due to a mechanistic mismatch with treatments targeting the established symptoms and not the pathophysiologic root cause. Therefore, an understating of the aetiology of hypertension is essential for the development of treatment and prevention strategies.

Current treatment strategies

Following decades of research into the disease, several major risk factors have been unveiled which can be classified into three categories: environmental, genetic and epigenetic. It is thought that the complex interaction between these factors are responsible for the development of hypertension. Hypertension can be classified as either primary, which is of unknown etiology, or secondary, which arise from known underlying renal, vascular, and/or endocrine diseases. It is estimated that 90-95% of adult cases of hypertension are of unknown etiology (3).

The initial approach taken to the management and treatment of hypertension involves lifestyle modification, including adjustment to diet, exercise, alcohol and tobacco consumption. Clinical trials indicate that dietary interventions that reduce saturated and total fat, that are rich in fruits and vegetables, and with abstinence of alcohol consumption and tobacco use can successfully lower blood pressure by as much as 11 mm Hg in individuals with hypertension (4). However, due to poor patient adherence to a strict diet and the complex and multi factorial nature of hypertension, a pharmaceutical approach is necessary in the majority of patients in the real-world environment.

Unfortunately, current pharmaceutical approaches have also had limited success. A number of studies indicate most patients fail to achieve systolic blood pressures below 140 mmHg (5-7). The proportion of patients achieving target blood pressures below 140/90 mmHg ranges from a maximum of 27% in the USA to a minimum of 3% in Zaire (8).

These issues associated with treatment may be circumvented by the development of novel anti-hypertensive agents. The development of such novel agents will be informed by a more comprehensive understanding of the aetiology and pathophysiology of hypertension.

BLOOD PRESSURE REGULATION

Over 90% of patients with hypertension receive a diagnosis of hypertension with unknown aetiology, also known as primary hypertension. This diagnosis is commonly followed by suboptimal therapeutic outcomes and reduced therapeutic compliance. An understanding of normal control of blood pressure can inform on the likely disease processes contributing to primary hypertension.

Indeed, in otherwise healthy people, blood pressure is tightly regulated to ensure constant perfusion of various tissues and to meet metabolic demands, while remaining low enough to avoid structural damage to tissues. Blood pressure regulation is a complex physiologic function involving multiple systems.

Local mechanisms act to regulate blood flow via the opposing actions of acute vasoconstriction and vasodilatation, and changes to the number and caliber of blood vessels supplying a tissue. Endothelial autocrine secretions play a vital role in inducing vasoconstriction and vasodilation. The renal-endocrine system is a chronic mechanism, controlling blood pressure over weeks and months via fluid balance and salt homeostasis; these mechanisms will not be discussed in this thesis as they are beyond the scope of the aims of this thesis.

In addition to these local mechanisms, global control of blood flow is mediated via the autonomic nervous system which can be divided into sympathetic and parasympathetic nervous system. The sympathetic nervous system is central to the control of blood pressure via direct adjustments to cardiac output and total peripheral resistance and indirect adjustments to blood volume via changes to renal function. The parasymapthic nervous system contributes mainly to the regulation of cardiac function.

Autonomic innervation of the heart

Although cardiac automaticity is intrinsic to pacemaker tissue, the heart is innervated by vagal (parasympathetic) and sympathetic fibers. Furthermore, heart rate and rhythm are largely under the control of the autonomic nervous system (9).

Postganglionic sympathetic neurons innervate the atria, ventricles and conduction system of the heart. The sympathetic modulation of the heart is mediated by the release of epinephrine and norepinephrine. Activation of β-adrenergic receptors upregulates cyclic AMP initiating protein kinase A mediated phosphorylation of membrane proteins and increases L-type calcium and pacemaker current (9). The end point is an acceleration of the slow diastolic depolarization resulting in an increase in heart rate (positive chronotropy), contraction of the myocardium (inotropy) and conduction velocity of atrioventricular node (dromotrophy).

The parasympathetic influence on the heart is mediated by cholinergic efferent vagal fibers, predominantly innervating the sinoatrial and atrioventricular nodes (10). However, there is some overlap in the anatomical distribution where the atrial muscle is also innervated by vagal efferent fibers and the ventricular myocardium sparsely innervated by vagal efferent. The release of acetylcholine by the vagus nerve inhibits hyperpolarization activated pacemaker current. The pacemaker depolarization results from a slow deactivation of the delayed rectifier current resulting in diastolic depolarization. Furthermore, acetylcholine acts on muscarinic acetylcholine receptors to increase cell membrane potassium conductance, slowing the speed of depolarization acting to slow the heart rate (9). Under normal resting condition, vagal tone prevails and heart period variations are mainly dependent on vagal modulation (11, 12).

Oscillations in heart period

Efferent sympathetic and parasympathetic activity to the sinus node is largely synchronous to each cardiac cycle and can be affected by central (vasomotor and respiratory centers) and peripheral (oscillations arterial pressure and respiratory movements) oscillations (13). These oscillations generate rhythmic fluctuations which manifest as short and long-term oscillation in the heart period and the analysis of these fluctuations may provide insight to the state and function of (a) the sympathetic and parasympathetic efferent activity, (b) the central oscillators, (c) the sinus node, and (d) humoral factors.

The modulatory effect of neural activity on the sinus node has been possible by spectral analysis of heart rate variability. Measures of heart rate variability have been utilized as tools to measure cardiac autonomic function in the studies represented in this thesis and methodologies will be covered in relevant chapters.

Autonomic innervation of the vasculature

In contrast to the heart, most arteries and veins receive only sympathetic innervation, while capillaries receive no innervation. Sympathetic adrenergic nerves tonically release norepinephrine activating α_1 and β_1 adrenergic receptors located on blood vessels. This tonic activation maintains vascular tone. The activation of vascular sympathetic nerves causes vasoconstriction of arteries and veins and contraction of vascular smooth muscles.

Baroreceptor reflex

The baroreceptor reflex is a rapid, homeostatic reflex which buffers fluctuations in blood pressure caused by everyday behaviors including stress, postural changes and physical activity (14, 15). Adequate buffering is crucial for regulating blood flow and maintaining gaseous and nutrient exchange with tissues.

Blood pressure changes are detected by mechanoreceptors known as baroreceptors located in the aortic arch and carotid sinus, Figure 1. These stretch receptors are activated when distended and send action potentials to the central nervous system which propagates signals through the autonomic nervous system. The end result is adjusting total peripheral resistance via vasodilatation and reducing cardiac output through negative chronotropic and inotropic regulation of the heart, Figure 2.

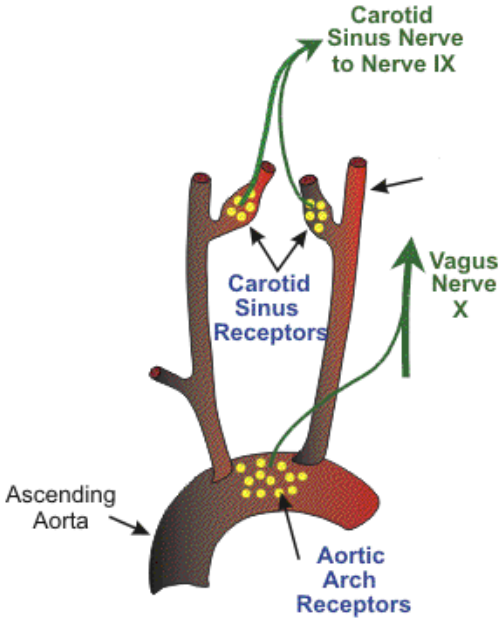
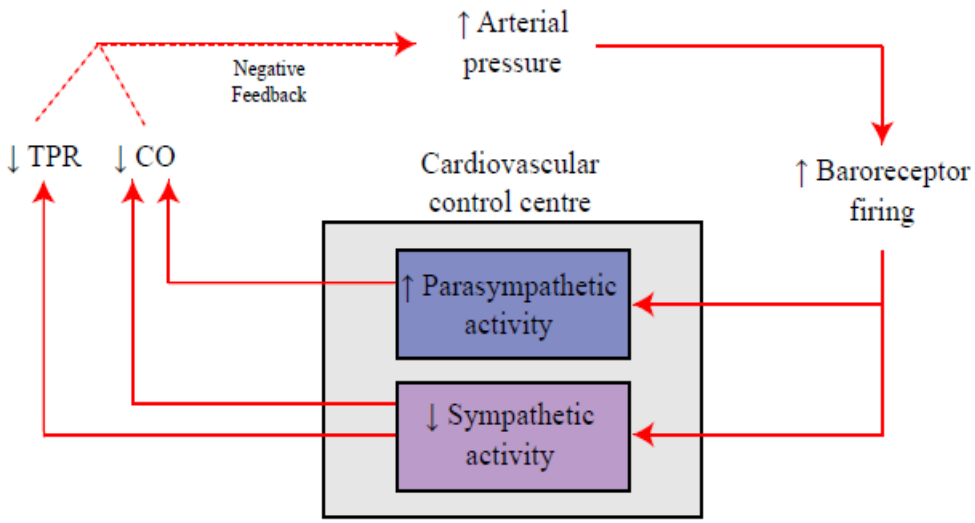


Figure 1. Location and innervation of arterial baroreceptors. Blood pressure changes cause a conformational change in baroreceptors resulting in changes to the neuronal firing. The Herine nerve and a branch of the glossopharyngeal nerve carries signals from the carotid baroreceptors and the vagal nerve carried signals from the aortic baroreceptors. Adapted from Klabunde, RE (16).



Haemodynamic formulae:

$$CO = HR \times SV$$

$$MAP = CO \times TPR$$

Figure 2. An increase in arterial pressure increases baroreceptor firing. Autonomic neurons within the cardiovascular control centre (medulla) respond by decreasing sympathetic nerve activity to the heart and blood vessels and increases parasympathetic activity to the heart, resulting in decreasing cardiac output (CO), heart rate, (HR), stroke volume (SV) and total peripheral resistance (TPR) ultimately reducing arterial pressure. Adapted from Klabunde RE (16).

NON-INVASIVE ASSESSMENT OF AUTONOMIC FUNCTION

The autonomic nervous system plays a key role in maintaining homeostasis by continually adjusting the function of organs in response to ongoing changes in the internal and external environment. Disruption to these homeostatic mechanisms may be a mechanistic contributor to the pathophysiology of disease.

The ability to reliably quantify dynamics of the autonomic nervous system is crucial for investigating diseases associated with autonomic dysfunction. Traditionally, the assessment of autonomic activity requires the use of invasive techniques such as measuring muscle sympathetic nerve activity using microneurography or measuring cardiac noradrenaline spillover to plasma using isotope dilution techniques. However, the use of these invasive methods limits their widespread use, particularly in otherwise healthy individuals including infants and children. The strong consensus that autonomic dysfunction may be pivotal in the development of hypertension has encouraged the development of quantitative non-invasive markers of autonomic activity.

In this thesis, autonomic function was assessed using the following techniques: (i) time and frequency analysis of heart rate variability, (ii) frequency analysis of blood pressure variability, (iii) spontaneous baroreflex sensitivity, (iv) baroreflex effectiveness index and a potential new marker of autonomic regulation of the myocardium (v) dP/dt_{max} variability of arterial pressure. These measures are all conducted post-hoc from electrocardiography and blood pressure waveforms.

Heart rate variability

Measures of heart rate variability (HRV) represent one of the most promising markers of cardiac autonomic function. ‘Heart rate variability’ is the conventionally accepted term to describe variations in both RR intervals and instantaneous heart rate.

Variations in heart rate can be evaluated using a number of methods. In the studies described in this thesis, HRV was calculated with time and frequency domain methodology. Details are provided in the relevant chapters, but in brief, key time domain measures included standard deviation of the NN interval (SDNN), and the square root of variance, an estimate of overall HRV (9). The square root of the mean squared differences of successive NN intervals (RMSSD) and the standard deviation of the difference between adjacent NN intervals (SDΔNN) as a marker of vagal modulation of the heart (17).

The autonomic nervous system mediates oscillations in heart rate and blood pressure at two main frequencies; high and low. Frequency domain analysis of HRV are determined by performing a fast Fourier transformation from an ECG trace, which enables the separation of the fluctuations into three pre-determined frequency components: very low, low and high frequency. The very low frequency ranges between 0-0.04 Hz, low frequency in the ranges of 0.04 to 0.15 with high frequency depending on the rate of respiration (18). Time and frequency analysis of heart rate variability has been used in a number of studies as a simple tool for both research and clinical studies (9), including those in infants and children with different phenotypes (19, 20).

Components of heart rate variability

Two frequency bands are shown to be related to autonomic function; low frequency reflects both sympathetic and parasympathetic modulation and high frequency reflects parasympathetic modulation to the heart (19, 20).

Efferent vagal activity contributes largely to the high frequency component. This is seen in clinical and experimental observations using autonomic maneuvers such as

muscarinic receptor blockage, electrical vagal stimulation and vagotomy (13, 21, 22). More debated is the interpretation of the low frequency component, considered as a marker of sympathetic modulation by some (13, 23-25) and by others, as a marker of both sympathetic and parasympathetic influence (21, 26). Consequently, the low to high frequency ratio is thought to be a marker of cardiac sympathetic balance (9).

The physiological basis of the very low frequency component is less well understood but may reflect changes in vasoactive hormone levels or thermoregulatory mechanisms (27).

It is important to recognize that heart rate variability measures autonomic modulations to the heart rather than the mean level of autonomic modulation. Therefore, autonomic withdrawal as well as a saturating high level of sympathetic modulation leads to diminished heart rate variability.

Blood pressure variability

Blood pressure can vary significantly over minutes throughout the course of the day, for example, changes in the beat to beat blood pressure variation in response to physiological or psychological stimuli, or in response to sleep and wakefulness. However, spontaneous blood pressure variations also occur independent of behavior (28). These fluctuations of blood pressure are the result of complex interactions between a number of mechanisms including mechanical influences of ventilation, cardiac vascular neural regulation, arterial stiffness, humoral and endothelial factors or genetic factors. However, when applied in the frequency domain, analysis can provide insight into integrated cardiovascular regulation (29).

Similar to heart rate variability, frequency analysis of beat to beat fluctuations in systolic blood pressure can be examined. The low frequency band of blood pressure

variability is regarded as an index of sympathetic modulation of the systemic vasculature and therefore total peripheral resistance (19, 30). The role of the high frequency component of blood pressure variaibility in autonomic regulation is less clear. However a recent study indicates that it may be linked to respiratory modulation of sympathetic vasomotor tone (31). Frequency analysis of blood pressure variability has been applied in a number of studies including the infant and child with different phenotypes (19, 32).

Baroreflex sensitivity

The sensitivity of the baroreceptor reflex is a measure of how much heart rate changes for a given change in arterial blood pressure. Traditional measures of baroreflex sensitivity are derived using invasive techniques, whereby vasoactive drugs are injected to produce changes in blood pressure, plotting the resultant heart rate or pulse interval response against blood pressure (33). More recently, the analysis of baroreflex sensitivity has been undertaken using computer-based techniques without the use of vasoactive drugs.

The sequence method scans the blood pressure wave form and identifies sequences of consecutive increases or decreases in systolic blood pressure followed by a progressive lengthening or shortening of heart rate or pulse interval over a period of four or more beats. This relationship is then plotted and fitted with a linear regression. The slope of the regression between systolic blood pressure and pulse interval or heart rate values in each sequence are indicative of baroreflex sensitivity (34).

Studies indicate that beat to beat changes in heart rate do not correspond to the fluctuation in systolic blood pressure in the same window, Figure 3. These delays are a result of different velocities of vagal and sympathetic regulation (35). Studies in infants indicate a delay of approximately two seconds for changes in heart rate in response to a change in blood pressure

(36), however no delays are found conversely in older children or adults. Therefore, the use of delays should be considered when identifying the relationship with corresponding heart rate or pulse intervals, and the relationship should be plotted for each delay in the neonate. Results obtained utilizing the sequence method are consistent with those obtained using traditional methods of vasoactive drugs. Previous studies have used the sequence technique to determine baroreflex sensitivity in the infant, child and adult (34, 36, 37). Similarly, this method was used in our studies to determine baroreflex sensitivity in the infant, child and adolescent.

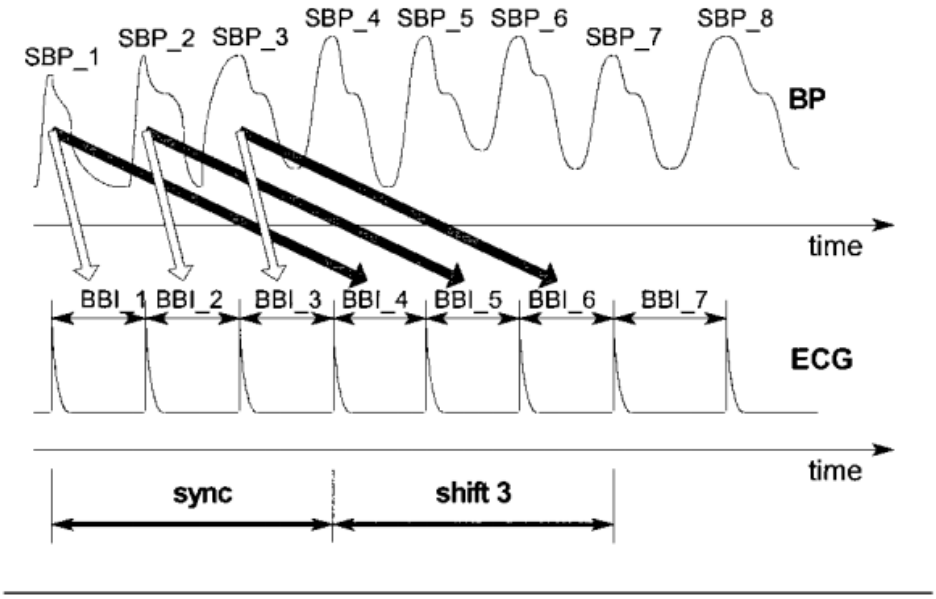


Figure 3. Visual representation showing beat to beat interval (BBI) does not respond to changes in systolic blood pressure (SBP) in the same window. Adapted from Malberg *et al* (35).

Baroreflex effectiveness index

In healthy individuals, the baroreflex is not always activated in response to every fluctuation in blood pressure. These sequences are interspersed with progressive systolic blood pressure changes that are not coupled with a reflex modulation. The presence of these uncoupled sequences may indicate that even in healthy individuals, the baroreflex may not be totally effective in inducing responsive changes to blood pressure fluctuations, despite being continuously involved in cardiovascular homeostasis (34).

This phenomenon can be quantified as the baroreflex effectiveness index and is determined as the ratio of the number of identified baroreflex sequences against the total number of systolic blood pressure ramps observed for a given period of time (34). It can be calculated using the formula below and may be useful in understanding baroreflex function between different phenotypes.

$$BEI = \frac{total\ number\ of\ PI/SBP\ sequences}{total\ numer\ of\ SBP\ ramps}$$

dP/dt_{max} variability

Estimated arterial dP/dt_{max} has been reported to be a surrogate measure for evaluating changes in left ventricular contractility (Rhodes et al., 1993). Although dP/dt_{max} of the arterial pressure waveform is affected by preload and arterial compliance (38), studies indicate a strong correlation between ventricular and arterial dP/dt_{max}. Furthermore, arterial dP/dt_{max} may offer a valuable methodology for non-invasive assessment of myocardial contractility (39-41).

A major influence on ventricular contractility is the autonomic nervous system (42). A study part of this thesis has introduced a possible new index of autonomic control of myocardial contractility, dP/dt_{max} variability. It is possible that sympathetic modulation of the ventricular myocardium may be reflected in the low frequency component of dP/dt_{max} variability, as the maximum rate of change in arterial pressure is related to the force of ventricular contraction. Accordingly, frequency analysis of dP/dt_{max} variability can be performed similar to systolic blood pressure variability, Figure 4.

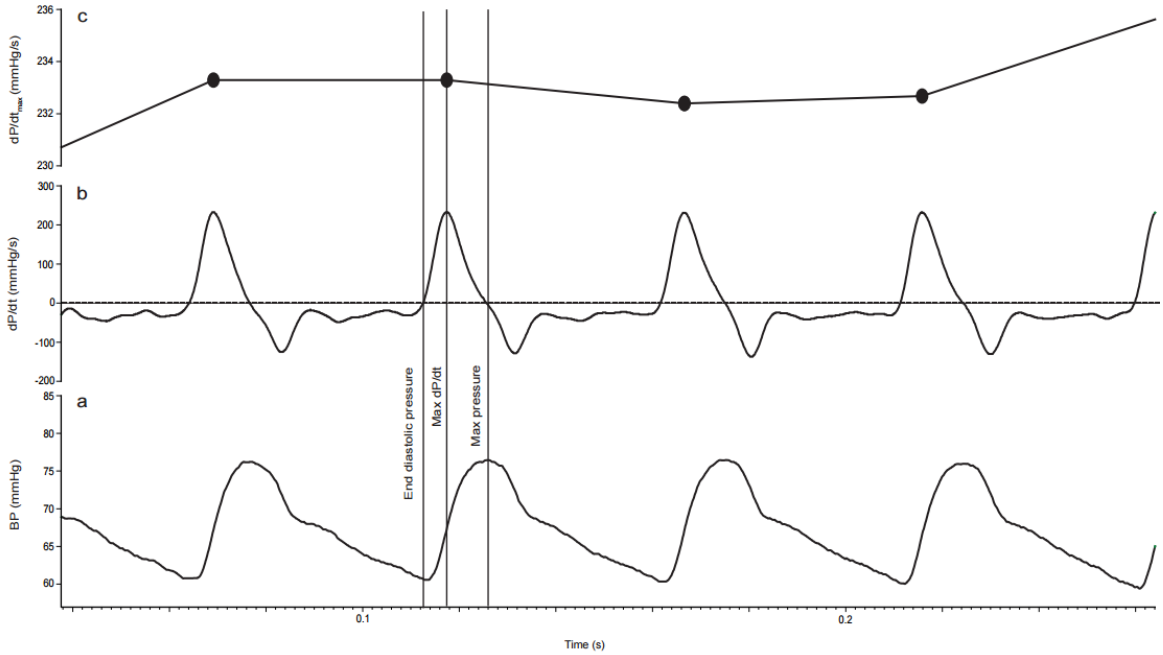


Figure 4: Example of blood pressure waveform **(A)** recorded in a control newborn **(B)** calculated dP/dt waveform and **(C)** dP/dt_{max} derived from the blood pressure waveform. dP/dt_{max} coincides with the maximum upstroke of the blood pressure waveform during systole. Applying frequency analysis over the dP/dt_{max} waveform enables the separation of fluctuations into individual frequency components.

AUTONOMIC ASSESSMENT DURING EXERCISE

Exercise testing has become a routine procedure in testing impairment of functional capacity in people with cardiac problems. Exercise is a form of physiological stress inflicted upon the body inducing a cardiovascular response. These tests can be useful in identifying individuals at risk of cardiovascular disease and interpretation of hemodynamic data. Furthermore, in some patients' cardiovascular disorders or the hypertensive phenotype may be unveiled only when challenged with an acute stressor.

In the context of programming, low birthweight children show decreased diastolic function and exercise induced cardiac fatigue after prolonged exercise (43) and very low birthweight premature children show limited functional capacity during exercise (44). However, other studies have found little differences in exercise performance between small for gestational age and appropriate for gestational age children (45). To date there are only a few studies that have investigated a cardiovascular response to exercise in those born small or large for gestational age.

Numerous exercise protocols and exercise machines have been used for paediatric and adolescent exercise testing. Each of these methods have their unique advantages and disadvantage. Cycle ergometry was used in our exercise testing protocol as our testing required an accurate blood pressure waveform and electrocardiogram and use of a bicycle is preferred over a treadmill for acquisition of these type of measurements (46). A disadvantage of using cycle ergometry is that not all subjects, especially in the paediatric population, are accustomed to cycling therefore premature muscular fatigue may prevent individuals from reaching their maximal effort. The complete exercise protocol used in this thesis for exercise testing is described in **Chapter seven**.

Cardiac autonomic function during dynamic exercise

The autonomic nervous system plays a crucial role in initiating cardiovascular responses to exercise. These changes ensure the increased metabolic demand is met. During exercise, there is a well characterised decrease in cardiac parasympathetic activity and an increased sympathetic activity to the renal, cardiac and splenic regions which results in an increased heart rate, cardiac output, stroke volume and helps redistribute blood flow to skeletal muscles (47).

The autonomic nervous system regulates cardiac function during exercise, these include regulation of cardiac contraction (inotropic), heart rate (chrontopic) and rate of myocardial relaxation (lusitropy) (48). In humans our understanding of cardiac autonomic control during exercise is mainly based on studies that have utilised heart rate variability analysis which has been validated in children and adolescents analysis (49-53) and or giving pharmacological antagonists that act on cardiac autonomic receptors (48). A number of studies have investigated the effect of exercise on heart rate variability, with both the intensity and duration of exercise being involved. Nevertheless, the literature indicates that on average heart rate variability is reduced (in time and frequency analysis) during exercise. With regard to sympathovagal balance, cardiac parasympathetic activity appear to be reduced during exercise, but normalised units of low frequency power (a measure of cardiac sympathetic and parasympathetic modulation) increases during low to moderate exercise and decreases during high intensity exercise (54-57). However, there are some conflicting results to these changes (58-60).

When utilising frequency analysis of heart rate variability during dynamic exercise it is important to consider the frequency ranges, especially the high frequency range. Typically, a high frequency band of 0.15-0.40 Hz is used, however during exercise this band may not be

suitable as higher respiratory frequency are observed during exercise. Therefore, different low (i.e 0.18 Hz) (61, 62) and upper limits (i.e 0.35, 0.50 or 0.80 Hz) (55, 58, 62) have been used during exercise.

Baroreflex function during dynamic exercise

The arterial baroreflex resets and functions around the prevailing heart rate and blood pressure during dynamic exercise (63-66). The resetting of the baroreflex is brought about by neural signals from higher brain centres and sensory afferents from active skeletal muscles (67). With the resetting of the baroreflex to a higher operating point, baroreflex sensitivity is reduced (68-71). This reduction is seen as the operating point of the baroreflex is shifted away from the point of maximum sensitivity, located at the centre of the baroreflex function curve, figure xx, (69). These changes are thought to occur due to an exercise induced reduction in cardiac parasympathetic activity (69). Collectively, measuring autonomic reactivity to exercise may provide some insight to autonomic stress reactivity in different phenotypes.

DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

The "Barker hypothesis"

The 'Barker hypothesis' posits that suboptimal *in utero* conditions may permanently alter the growth and maturation of fetal tissue, leading to long-term changes in structure and physiological function that may influence risk of disease in later life(72-74). This concept is commonly known as fetal programming and is a core component in the field of developmental origins of health and disease.

This concept arose from epidemiological observations made by Barker and colleagues which showed geographical distribution of infant mortality in England closely matched the distribution of death rates from coronary heart disease decades later (75). The most commonly registered cause of infant death during this time was low birthweight. This observation led to the hypothesis that individuals with a low birthweight who survived infancy and childhood in those regions may be at an increased risk of coronary heart disease later in life.

Following this initial observation made by Barker and Osmond, a number of other studies have found evidence consistent with this hypothesis, showing a strong association between low birthweight and increased risk of later cardiovascular disease (75-81).

Fetal growth and adult cardiovascular disease: a life course perspective

Impaired fetal growth due to maternal malnutrition or placental insufficiency is a well-established risk factor for increased cardiovascular morbidity and mortality in adulthood (82). Reduced fetal growth is frequently accompanied by systemic growth restriction resulting in changes to the structure and function of key organs which control blood pressure. These adaptive changes prepare the infant to similar conditions postnatally, ensuring its survival, giving rise to a thrifty phenotype. If these individuals are exposed to a high caloric diet in postnatal life, they may be at an increased risk of developing metabolic disorders such as type II diabetes and abdominal obesity. Indeed, a number of studies describe an association between low birthweight and higher risk of hyperlipidemia, type 2 diabetes and greater central distribution of fat (72, 83, 84), all risk factors for cardiovascular disease.

Animal models provide further evidence for an association between fetal growth restriction and adult cardiovascular disease. A number of prenatal manipulations including maternal under nutrition, glucocorticoid exposure, hypoxia and placental obtrusion show cardiovascular programming in the rat, sheep, pig and guinea pigs (85-87).

Since the observations made by Barker and colleagues, this field of research have evolved to encompass a number of other environmental insults in addition to undernutrition.

With the growing obesity epidemic, attention has recently been directed towards maternal over nutrition. There is accumulating evidence in humans that those born large for gestational age may also be at increased risk of developing cardiovascular and metabolic disease in later life. For example, those born large for gestational age show increased systolic

blood pressure and obesity as children (88-91) as well as adverse cardiometabolic health as children and adults (92-94).

Furthermore, there is accumulating evidence from a number of animal studies supporting the hypothesis that increased maternal weight, obesity or a high fat diet during pregnancy is associated with obesity and hypertension in the offspring (95-100).

Collectively, it is becoming increasingly evident that those born small or large for gestational age may be at increased risk of later cardiovascular and metabolic disease. However, it is important to understand that although programming of cardiovascular disease can begin *in utero*, programming can also occur in postnatal life, particularly during infancy while plasticity of organs and tissues remains high. This can occur due to premature births (101-103), growth trajectories (104-107) or changes in nutrition composition (108, 109).

Therefore, given the high prevalence of cardiovascular disease, targeting established cardiovascular disease in the adult is no longer effective in ameliorating the growing burden. It is therefore important to consider a life course perspective, an approach based on the premise that the aetiology of chronic diseases begins decades prior to their clinical presentation, and that they can be prevented and controlled at multiple stages of the life course. This requires an understanding of the behavioral, biological and psychosocial processes across the life course of an individual (110), in order to enable interventions to be developed and implemented to prevent clinical disease outcomes.

Developmental plasticity is highest in early life and declines over the life course which results in increased susceptibility to disease due to a reduced ability to adapt to new stimuli, Figure 5. Although the greatest risk of disease is in adulthood, risk factors for

diseases accumulate over an individual's life course. Identifying these early risk factors and phenotypes are imperative and enables risk stratification and the development of nutritional and lifestyle interventions. A number of maternal factors including dietary intake, body composition and stress can affect fetal development. These changes encountered *in utero* may predispose the exposed individual to later cardiovascular risk. Similarly, nutrition and development during infancy and childhood will influence risk of disease at a later stage. Therefore, early intervention, such as during early childhood, may have a greater impact on reducing disease risk than interventions implemented later in life, figure 5.

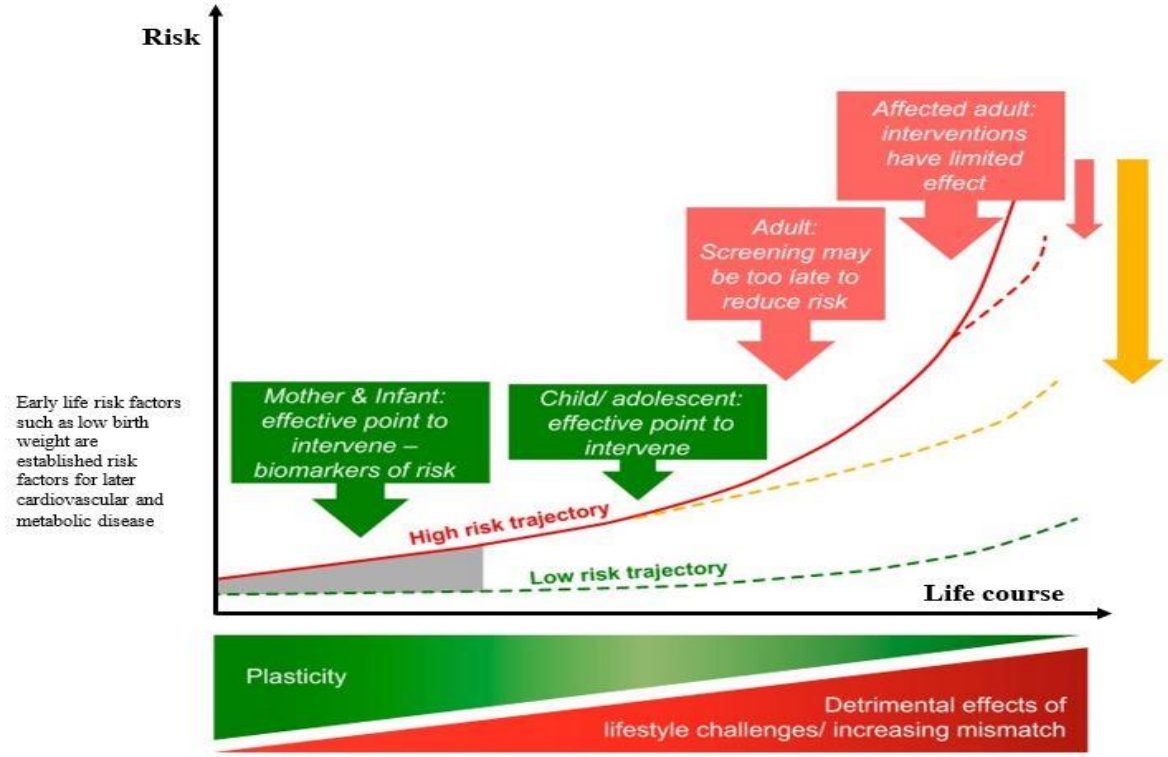


Figure 5. Life course perspective of disease risk. Nonlinear increase in disease risk as plasticity decreases and accumulative effects of adverse stimuli over the life course. Early intervention reduces risk trajectories. Adapted from Hanson *et al* (111).

Fetal growth and adult cardiovascular disease: plausible mechanisms

As described in the previous section, there is a strong association between birthweight and risk of adult cardiovascular disease. However, it is unlikely that birthweight itself is the cause of increased cardiovascular risk, but simply a crude marker of other process that are occurring. To date, it is unclear whether developmental alterations in the kidney, vasculature or the brain causes increased risk of cardiovascular disease in those born small or large for gestational age. A number of studies show altered structural and functional changes to the kidney and vasculature in programming studies.

Early studies focused on the kidney as a key site for programming of hypertension. In humans, low birthweight is accompanied by a reduced nephron number (112, 113). These studies provide a plausible evidence-based mechanism linking low birthweight with poor blood pressure control, and subsequent hypertensive disorders. Other studies, however, have failed to demonstrate a link between reduced nephron number and hypertension (114, 115).

Vascular function studies in low birthweight individuals show decreased vascular compliance (116), endothelial dysfunction (117), impaired endothelial-dependent and independent vasodilation at birth, 3 months of age, childhood and adult life (118-120). Low birthweight individuals also show structural changes to the vasculature. These include; increased arterial and large artery stiffness (121), increased aortic wall thickening (122), reduced compliance and aortic size (123), carotid atherosclerosis (117, 124) and narrowing of coronary arteries (125). More recently, young adults born large for gestational age show increased carotid intima-media thickness, a marker of subclinical atherosclerosis (92) as well as newborns (93).

AUTONOMIC NERVOUS SYSTEM AND PROGRAMMING OF HYPERTENSIVE DISEASE

Animal models

Experimental studies with animal models have played an important role in investigating the roles of maternal nutritional manipulations and placental insufficiency in driving alterations to autonomic activity as a mediator of the increased blood pressure in adulthood (126-132).

Studies looking at a maternal low protein diet found alterations in catecholamine metabolism in 12-week-old rat offspring from mothers fed a low protein diet during pregnancy and lactation, consistent with an increased sympathoadrenal activity during the fed state (133). A study designed to measure renal sympathetic nerve activity in protein restricted rats found increased renal sympathetic nerve activity in response to stress and not during resting conditions.

Studies looking at neuronal activity have shown increased levels of corticotropin-releasing hormone RNA expression in the paraventricular nucleus (PVN) of humans with primary hypertension (134), in spontaneously hypertensive rat (135), and in the hypothalamus of hypertensive rats programmed via maternal undernutrition (136). These studies indicate a possible role of the PVN in the pathogenesis of hypertension in humans and in animal models. The PVN is a neuronal nucleus located in the hypothalamus, which is activated in response to stress and physiological changes. Neurons projecting from the PVN have important functions in regulating the autonomic nervous system. A study by Navarrete and colleagues (2007) investigated the excitatory connections of the PVN neurons between offspring from mothers given a restricted diet and those from a control diet found, offspring of undernourished mothers

exhibited increased neuronal activity in the PVN and locus coeruleus with an increased systolic pressure compared to their control counterparts. Furthermore, they tested the effect of microinjecting α_1-adrenoceptor antagonist prazosin into the PVN and corticotropin-releasing hormone into the locus coeruleus. Microinjecting prazosin into the PVN decreased neuronal activity and systolic blood pressure in the undernourished offspring; whereas microinjection of corticotropin-releasing hormone into the locus coeruleus stimulated neurons and increased systolic blood pressure in control rats. Taken together, two alternatives are possible for the effects observed; (i) desensitization of corticotropin-releasing hormone receptors due to hyperactivity in undernourished offspring and/or (ii) neurons are already fully active in the undernourished offspring and are insensitive to any further excitation via exogenous corticotropin-releasing hormone (136). This study points to the possibility of a hyperactive PVN- locus coeruleus loop in offspring of mothers given an undernourished diet. This hyperactive loop could potentially be responsible for increased stimulation of the sympathetic nervous system and therefore, the hypertensive state of these undernourished rats (136).

Initial studies directed their focus on maternal/fetal under-nutrition and low birthweights. However, in light of the obesity epidemic, attention has recently been shifted to the role of maternal over-nutrition in programming of hypertension. There is now substantial evidence from animal studies supporting the hypothesis that maternal overweight, obesity or high fat dietary intake is associated with obesity and hypertension in the offspring (137-140). Furthermore there is evidence suggesting raised sympathetic activity may be mechanistically involved in programmed hypertension (137, 141). A study by Samuelsson et al (137), showed hypertension and increased sympathetic activity in juvenile rat offspring (30 days of age) and as young adults (90 days) from mothers fed an obesogenic diet during pregnancy. Moreover, administration of α and β-adrenergic blockers reduced blood pressure to levels comparable to

control rats. In addition, these offspring developed hypertension and increased sympathetic activity as juveniles, prior to the onset of overt obesity. Therefore, this suggests changes to autonomic activity and hypertension arise as a direct consequence of *in utero* exposure to maternal obesity. A study by Rudyk and colleagues (141), determined the effects of a high fat diet in the absence of maternal obesity on cardiovascular and autonomic function in rats during rest and in response to an acute stressor. Resting blood pressures were similar between groups although offspring from mothers fed a high fat diet showed heightened cardiovascular response to stress with a prolonged recovery period. Following salt loading, these rats showed a heightened nocturnal blood pressure and an increased low/high frequency ratio of HRV. This suggests that a high fat diet during pregnancy, in the absence of maternal obesity, results in altered sympathetic control and hypertension in the offspring, secondary to an acute stress response (141).

Similarly, a study in rabbits investigating the effects of a maternal high fat diet on blood pressure and renal sympathetic nerve activity found that offspring from a maternal high fat diet had higher MAP, HR and renal sympathetic nerve activity at four months of age compared to their control counterparts. Furthermore, in response to an acute stressor, the high fat offspring exhibited an exaggerated sympathetic response. However, it is difficult to dissociate whether the increased sympathetic activity seen in the high fat offspring are due to excess visceral fat found in these offspring, or due to the direct programming of the sympathetic nervous system due to a maternal high fat diet (142).

In addition to the increased consumption of diet high in fat, there is also a growing trend in the consumption of beverages high in sugar. Despite the known adverse effects of high sugar beverages on cardiovascular and metabolic health, their effects on the developing fetus have

not been studied extensively. In pregnant mice, obesity induced by feeding a diet rich in animal fat and sugars resulted in hypertension and increased fat mass in the offspring (143). The same investigators subsequently examined the effects of a maternal diet with high sugar content in isolation and found that it induced significantly higher blood pressures in both male and female offspring at three months of age (144). Furthermore, both male and female offspring showed increased LF:HF ratio in their HRV and raised renal noradrenalin, suggesting that the hypertension may be due to increased sympathetic tone.

Studies in Humans

Chapter two of this thesis details studies investigating the autonomic nervous system and programming of hypertension in humans.

PLACENTAL FUNCTION

The placenta is a key organ that connects the growing fetus to the uterine wall and facilitates the transfer of nutrients, gas exchange, waste elimination, and thermoregulation. In addition, the placenta metabolises a number of substrates, produces key hormones that affect pregnancy, fetal growth and metabolism, as well as protect the fetus from infection and maternal diseases (145). These different functional properties of the placenta can be categorized into different systems. These include transport and metabolism, protection and endocrine. Any functional changes to the placenta directly affect the fetus, altering its ability to adapt and respond to the intrauterine environment, ultimately affecting fetal growth (145).

The Placenta and programming of disease

The developmental origins of health of disease concept involves early environmental exposures and other influences during gestation, and their links with postnatal predisposition of an individual to disease in later life. The placenta plays a key role in these processes and it is possible that the links between nutritional and endocrine factors with later disease are mediated via placental health and control mechanisms. The evidence linking the placenta to later disease is based on placental weight (146). Placental weight is strongly correlated with the weight of the newborn, and as fetal weight increases throughout gestation, so does placental weight.

Several studies have shown that placental weight is associated with increased risk of hypertension in later life (82, 146, 147). However, studies in children show inconsistent associations with blood pressure (148). Moreover, studies report an association between increased blood pressure levels with low and high placental weight in relation to birthweight (149, 150). However, placental weight is directly associated with the placental surface area

available for nutrient and oxygen exchange. Studies in animals indicate that if ewes are undernourished during mid pregnancy, the placenta can expand, thereby increasing its surface area allowing for increased nutrient exchange from the mother (151, 152). However, these changes were only seen in those who were well nourished at time of conception. A study including 2003 subjects found reduced placental weight and area to be associated with hypertension but not reduced placental thickness (148).

Fetal nutrition depends partially on maternal nutrition, metabolism and maternal nutrient stores. A number of studies indicate maternal factors such as diet, body composition, body mass index, maternal blood glucose and lifestyle influence placental size and therefore fetal development (148, 153, 154). Maternal factors may affect fetal development via the placenta by modifying placental metabolism and nutritional transport or, independent of placental tissue (i.e. nutrients enter the fetal circulation directly) (154). Several studies indicate that both maternal and fetal factors affect transport capacity, ultimately altering placental function and amount of glucose, amino acid and fatty acid transported to the fetus (154-156).

Early life inflammation

More recently, infection and early life inflammation, which can affect the placenta, have been proposed to contribute to increased cardiovascular risk. Many of the early life risk factors such as poor intrauterine growth, maternal obesity, diabetes, hypercholesterolaemia and smoking are associated with an increased inflammatory state (157). Moreover, inflammation plays a central role in the development of atherosclerosis and cardiovascular disease, and therefore, early life inflammatory events and infection may predispose these individuals to increased cardiovascular risk.

To date, there are a limited number of studies that have investigated the role of perinatal inflammation and infection on cardiovascular risk. A perinatal inflammatory exposure most commonly elicited in response to an infection within the uterus during pregnancy can result in a maternal (chorioamnionitis) and fetal inflammatory response, both of which can be histologically identified within the placenta (158). It is possible that a perinatal infection or inflammatory exposure may affect developing organs such as the heart and blood vessels (159), contributing to pathways underpinning epidemiological associations between poor intrauterine growth and adult cardiovascular disease.

Animal models of chorioamnionitis show associations between chorioamnionitis and hemodynamic changes (157), with reduced cardiac function (160), impaired myocardial contractility and relaxation (161), increased cardiac afterload and reduced cardiac output in fetal mice (162), reduced ventricular contractility and reduced cardiomyocyte numbers in fetal sheep (163).

In human newborns, increased abdominal aortic intima-media thickness, a marker of preclinical atherosclerosis, is associated with increased inflammatory mediators in the amniotic fluid (164) and in maternal (165) and umbilical cord plasma (166). A study in preterm infants showed that histological chorioamnionitis is associated with increased serum high-sensitivity C-reactive protein during their first week after birth, and a non-significant trend towards an adverse lipid profile. However, measures of aortic intima-media thickness and blood pressure in these infants showed no association with chorioamnionitis. The short duration and the late on set of chorioamnionitis later in the gestational period may be insufficient to induce changes to arterial structure. Nevertheless, follow up studies of these infants may unveil altered risk trajectories (167).

Collectively, altered placental function via maternal factors or a perinatal inflammatory exposure may affect fetal growth and development and predispose the individual to later cardiovascular and metabolic risk. However, the mechanisms which ultimately mediate these changes are largely unknown.

BIRTHWEIGHT PHENOTYPE

Normal fetal growth is imperative for the immediate and long-term health outcome of the offspring. To determine those born small or large, reference values for birthweight for gestational age and gender are commonly used. These population references provide birthweight percentiles for each gestational age with birthweights below the $<10^{th}$ percentiles defined as small for gestational age (SGA), those with birthweights from 10^{th} to 90^{th} percentiles defined as appropriate for gestational age and, those with birthweights $>90^{th}$ percentile defined as large for gestational age (LGA). Those born small or large for gestational age are at increased risk of developing cardiovascular disease with both groups showing altered metabolic function as infants, children and adults. (168). The underlying mechanism driving disease progression in these two distinct groups are likely different.

Birthweight and birthweight percentiles have long been used by both researchers and clinicians to determine at risk individuals. As described extensively, early studies found birthweight as a predictor of infant mortality, with a 'U-shaped' association (see figure 6) (169).

However, the use of birthweight percentiles has some inherent problems. Firstly, there are a number of maternal variables that have an independent effect on birthweight. These include parity, ethnicity, weight and height, all of which program a unique predetermined growth potential in the fetus (170). Birthweight percentiles do not take these into account and therefore, does not discriminate well between a baby who is just constitutionally small who have met their unique growth potential, from a baby who is pathologically small who did not reach their unique growth potential. Similarly, babies with excess growth above their expected growth trajectory may be pathologically large. Distinguishing babies who are

pathologically small or large from "normal" is imperative as they require different types of care and using a single birthweight percentile does not differentiate between these babies.

Second, the quality of growth is important as two babies of the same sex may have the same birthweight but have a very different body composition. Available fat stores in the baby are crucial for delivering an energy supply post birth especially prior to establishment of breastfeeding (171). Those infants categorized as "normal" based on their birthweight, but who have failed to reach their growth potential and have low fat content, may be a group that would specifically benefit from body fatness assessment in the early postnatal period.

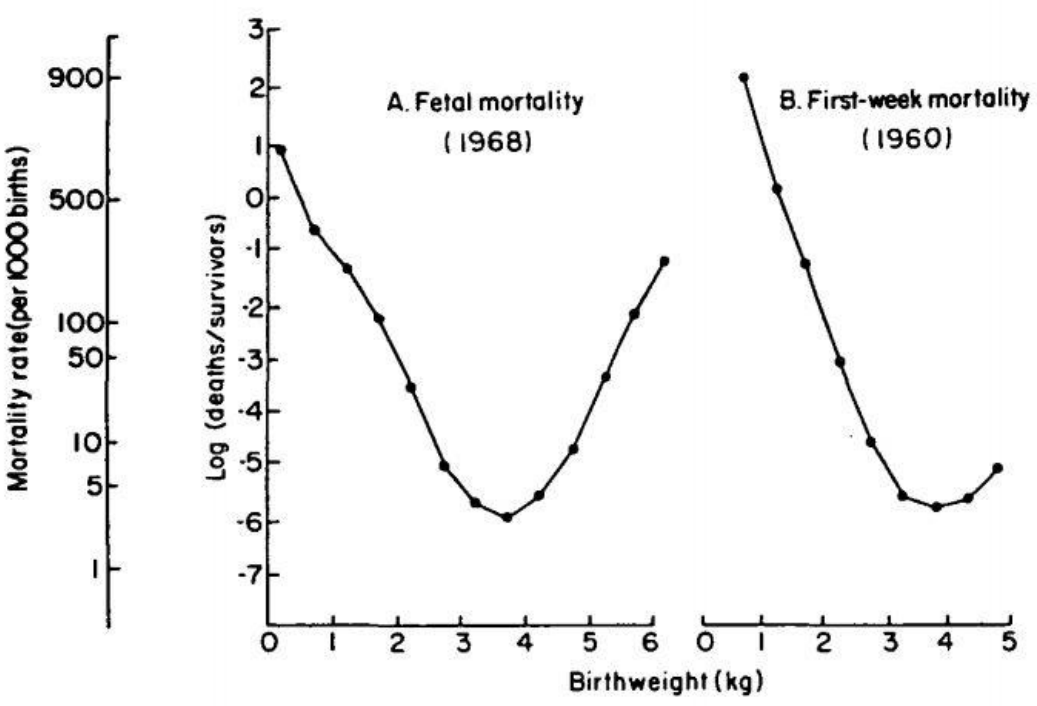


Figure 6. Birthweight-specific mortality curves. Fetal and infant mortality is highest at the lowest birthweights but falls sharply as birthweight increases and rises again for those with the highest birthweights. Adapted from Wilcox AJ *et al* (169)

Newborn body composition

Body composition describes the percentage of muscle, fat, bone and water in an individual. Therefore, two people of the same weight and sex may look very different as different type of tissue in the body take up different amounts of space in the body. Muscle tissue takes up less space than fat tissue, therefore leanness is determined by both weight and body composition.

Body composition is a key component of health and it is realized that several aspects of body composition especially percentage body fat and the amount of lean mass are important indicators of health outcomes and physical status in infants, children and adults. A study conducted at the Royal Prince Alfred Hospital, Sydney, showed that percentage body fat was a better indicator of neonatal morbidity compared to birthweight (172).

There are several techniques available to measure body composition. These include dual-energy X-ray absorptiometry, hydrostatic weighting, measurement of total body water using isotope dilution as well as multicompetent models (173). However, these techniques have practical limitations as they are time consuming, invasive and may involve some radiation and therefore cannot be easily applied in a neonatal or child population.

The commercially available system known as the PEA POD (COSMED, Concord, CA, USA), utilizes the air-displacement plethysmography technique to measure body composition. The use of the PEA POD offers a number of advantages over other measures as it is a fast, automated, safe, noninvasive measure and can be applied in the neonate (174)

Briefly, air displacement plethysmograph uses whole body densitometry to determine body composition. Using the pressure and volume relationship of Boyles Law, the volume of the neonate situated inside a chamber is derived. Using measures of body volume and body mass permits calculation of body density (175). Once whole-body density is known, estimates of percentage fat, fat mass and fat free mass can be determine using an equation such as those derived by Siri (176):

Percent Fat = 495/density – 450

These equations are integral to the software of the device. Mass of the neonate is easily obtained using the integrated electronic scales within the PEA POD measured to the nearest 0.1g (175). The PEA POD device has been validated in the neonate, and is the a quick noninvasive method for body composition assessment in infancy (174)

Measures of body composition, fat mass and lean body mass utilizing the PEA POD have refined the assessment of fetal growth. This has enabled body fat percentiles charts to be created according to gestational age and gender, table 1 (177). Infant body composition measurements are now routinely conducted as part of general care at the Royal Prince Alfred Hospital, Sydney.

Centile	36–37⁶⁄₇ wk Gestation			38–39⁶⁄₇ wk Gestation			40–41⁶⁄₇ wk Gestation		
	Male	Female	All	Male	Female	All	Male	Female	All
97.5th	14.9	17.5	17.1	19.0	18.2	18.4	18.2	22.1	19.8
95th	14.5	16.9	14.4	17.1	17.7	17.5	16.2	19.2	18.3
90th	13.0	13.1	12.9	14.5	16.3	15.5	15.0	17.9	16.7
75th	11.9	12.0	11.9	12.2	14.1	13.0	12.7	15.4	14.2
50th	9.2	8.9	9.2	9.6	11.0	10.3	9.9	12.5	10.9
25th	6.0	5.7	5.9	7.2	7.9	7.5	6.9	9.4	8.1
10th	4.6	4.0	4.4	4.7	6.2	5.1	4.9	7.2	5.8
5th	3.4	1.8	3.3	3.2	4.7	3.4	3.4	5.6	4.4
2.5th	3.1	1.4	1.7	2.4	2.9	2.6	2.8	4.7	3.2

Table 1: Newborn body fat percentiles according to gestational age and sex, adapted from Hawkes CP *et al* (177).

Child and adolescent body composition

Over the past four decades there has been a tenfold increase in the number of obese children and adolescents (aged five to 19 years). Worldwide, this increase was from 11 million in 1975 to a staggering 124 million in 2016 (178). Furthermore, an additional 213 million were classified as overweight in 2016 (178).

Children as young as 3 years of age who are overweight show increased inflammatory markers compared to those with normal weight (179). Hypertension, dyslipidemia and dysglycemia are already seen in children with obesity. Furthermore 19% to 38% of these children also present with the metabolic syndrome (180, 181). This increasing trend of obesity among children and adolescents poses a great risk for the long-term health of these individuals as obesity tracks strongly, such that approximately 70% of 6-9 year old children with obesity will have obesity as adults (177).

Currently, body mass index (BMI) is widely used to identify children and adolescents with high adiposity, those between the 85^{th} and 95^{th} percentile (sex and age adjusted) are classified as being overweight and those with a BMI greater than the 95^{th} percentile are classified as being obese (182). Similar to birthweight percentiles, BMI cutoffs are population based and may not reflect individual growth patterns. A study investigating the use of BMI in the prediction of cardiovascular disease risk factor clustering in a biracial sample of children and adolescents found, identifying risk of metabolic syndrome for a given BMI differed significantly between white and black obese adolescents (183). Furthermore, children and adolescents may also be classified as having a normal weight and BMI, but may in fact have increased adiposity which increases their risk of cardiometabolic diseases. These limitations associated with the use of BMI have led to the use of body composition

measurements as a tool to accurately identify individuals at risk of developing obesity-related disease.

The BOD POD (COSMED, Concord, CA, USA) is an air displacement plethysmograph, similar to the PEA POD but validated for assessment of body composition in older children and adults(173, 184-186).

AIMS

Birthweight is associated with hypertension across the lifespan and adult cardiovascular disease, such that those at both ends of the spectrum are at increased risk. Nonetheless, birthweight is a relatively crude surrogate of fetal growth and it may be that quantification of body composition, particularly body fatness, may more accurately identify the “high risk” individual. Causative mechanisms linking birthweight and risk of cardiovascular disease likely involve changes to the structure and function of organs including the placenta, which may impair development and predispose individuals to later cardiovascular disease.

The aims of this thesis were to investigate the association between placental function, body composition and cardiovascular autonomic function. To achieve these aims, the following associations were tested in three settings:

1. the relationship between maternal factors, placental factors, inflammation and infant body composition
2. the associations of infant body composition and late preterm birth with cardiovascular autonomic function
3. the association of body composition with autonomic functions in the child and adolescent.

CHAPTER TWO

AUTONOMIC DYSFUNCTION IN PROGRAMMED HYPERTENSION: A REVIEW

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In preparation for journal submission

ABSTRACT

Hypertension is an important modifiable risk factor for cardiovascular and cerebrovascular diseases. Its high prevalence, combined with the morbidity and mortality associated with secondary complications, make it a major public health concern. Despite decades of research, over 95% of all cases of hypertension remain of unknown etiology, necessitating that treatments target the established symptoms and not the cause. One of the important recent advances in hypertension research is an understanding that hypertension often may have a developmental origin. A substantial body of evidence indicates that exposure to an adverse intrauterine environment during critical periods of development may predispose an individual to develop hypertension later in life. A causative mechanism has yet to be identified, but may include epigenetic modifications, and/or alterations in renal, vascular or autonomic cardiovascular functions. This review will present evidence regarding changes in autonomic activity as a possible causative pathophysiological mechanism underlying the development of programmed hypertension.

In man, low birth weight is the best-known risk factor for hypertension of developmental origins, although this is a broad surrogate measure for intrauterine adversity. This review will include clinical studies across the lifespan that have investigated autonomic function in groups that include; individuals with fetal growth restriction and those born preterm. A determination of whether altered autonomic function is seen in these individuals in early life is imperative, as hypertensive disorders that have their origins in utero, and that can be identified early, will open the door to risk stratification, and the development of new strategies that prevent or specifically target these mechanisms.

INTRODUCTION

Cardiovascular and cerebrovascular diseases are a major cause of morbidity and mortality worldwide. According to the most recent reports from the World Health Organisation (1), 15 million people died from cardiovascular diseases in 2015; estimated to rise to 23 million deaths annually by 2030 (2). Hypertension is an important modifiable risk factor for cardiovascular and cerebrovascular diseases (3), affecting approximately 40% of people (2). In total, hypertension accounts for 9.4 million deaths worldwide every year (2). The World Health Organisation estimates that globally hypertension is responsible for at least 51% of deaths due to stroke and 45% of deaths due to heart disease. In the USA, it is estimated that the health care costs attributed to hypertension in 2010 were in excess of 90 billion dollars (4) .

Successful treatment of hypertension remains poor, and it has been argued that this is partly due to a mechanistic mismatch, with treatments targeting the established symptoms and not the upstream pathophysiologic root cause. Despite decades of research into its aetiology, an important proportion of hypertension remains categorised as “essential” hypertension, i.e. without unknown cause(5) . A meaningful proportion of essential hypertension may have neurogenic origins, in particular relating to sympathetic nervous system activity.

A substantial body of epidemiological and experimental evidence indicates that exposure to an adverse intrauterine environment during critical periods of development may predispose an individual to develop cardiovascular disease later in life. Indeed, it has been argued that a meaningful proportion of cases of primary hypertension may have a programmed or developmental origin (6, 7), with putative

mechanisms including epigenetic modification, changes to nephron number, glucocorticoid levels, endothelial dysfunction, activation of the renin-angiotensin system (RAS), and autonomic dysfunction (8). This review will present evidence regarding autonomic activity as a possible causative pathophysiological mechanism underlying the development of programmed hypertension.

AUTONOMIC NERVOUS SYSTEM AND HYPERTENSION

The autonomic nervous system has important functions in the control of blood pressure via direct regulation of cardiac output and total peripheral resistance, and indirect regulation of blood volume via alterations to renal function. A key characteristic of hypertension, a major contributor to most cardiovascular diseases, is elevated sympathetic nerve activity (9). Raised sympathetic nerve activity is established during childhood in some humans prior to the development of clinical hypertension. Moreover, evidence for raised sympathetic activity has been observed in normotensive subjects considered at increased risk of developing hypertension, such as children with a familial history of hypertension (10), those with borderline hypertension, and also in those with white-coat hypertension (11-13), suggesting that raised sympathetic activity precedes clinical hypertension, and supporting a causal role of sympathetic dysfunction in the etiology and pathophysiology of hypertension. Children with a family history of hypertensive disorders also exhibit decreased baroreflex sensitivity (10), a related yet distinct, pathway consistent with a predisposition to developing hypertension. Furthermore, there is convincing evidence from studies measuring noradrenaline spillover and using direct neural recordings that raised sympathetic nerve activity is present in both overt hypertension and in borderline hypertension (11, 14-17). Such “neurogenic hypertension” has been

reported to account for at least 50% of all cases of high blood pressure (18). Studies also suggest that essential hypertension, and particularly resistant hypertension, is characterized by raised sympathetic cardiovascular drive (19-21), with both showing pronounced sympathoinhibition in response to anti-hypertensive interventions. On average, people with hypertensive disorders also have lower cardiac parasympathetic nerve activity (22) (23). Taken together, these data indicate that there is a strong neurogenic component associated with the pathophysiology and etiology of hypertension.

These clinical studies are supported by evidence from experimental studies, in particular those utilizing the spontaneous hypertensive rat (SHR), which have been important in establishing a causal link between sympathetic over-activity and hypertension. Jude and colleagues (1976), (24) showed this link in early studies, where, sympathetic nerve activity and blood pressure were shown to increase rapidly as SHRs aged, and by five weeks of age, both blood pressure and sympathetic nerve activity were significantly higher than values observed in normotensive strains of rats (24). When sympathetic ganglionic transmission was reduced via hexamethonium administration in SHRs, both sympathetic nerve activity and mean arterial pressure were reduced to a level comparable with normotensive controls (24).

AUTONOMIC NERVOUS SYSTEM AND PROGRAMMED HYPERTENSION

The mammalian nervous system begins its development during fetal life and continues after birth. Accordingly, both intrauterine and postnatal exposures may affect and alter the development of neural components. There is a wealth of

epidemiological evidence indicating raised blood pressure can be influenced by in utero exposures (8), specifically an inverse association between birth weight and risk of hypertension and cardiovascular disease in later life, such that those with the lowest birth weight are at highest risk of these cardio-metabolic diseases (25, 26). In particular, those with fetal growth restriction, including mild to moderate forms, are at the highest risk of heart disease and type 2 diabetes, whilst those born preterm birth have evidence of raised blood pressure (27, 28). These infants are exposed to distinct early life exposures that may infer risk via distinct pathways, including reduced time to develop prior to birth, and/or insufficient nutrient availability due to malnutrition or impaired utero-placental perfusion. To better understand these underlining mechanisms, it is important to understand whether altered autonomic function is due to prematurity or growth restriction alone and whether there is an additive effect in those born premature with low birthweight.

Evidence for altered autonomic function in individuals born SGA or growth restricted

A number of studies have investigated the relationship between fetal growth restriction and altered autonomic function, from the perinatal period through to childhood and adult life. However, studies in the term born, growth restricted infant is limited, a study by Galland and colleagues 2006, found increased sympathetic activity and reduced tachycardiac response to head-up-tilt test at 1-3 months in infants born small for gestational age (29). This association appears to persist into adolescence and adulthood. Amongst twins, the duration of the cardiac pre-ejection period, a marker of sympathetic control of heart rate (30, 31), was shorter in the twin with lower birth weight (30); accounting for 63-84% of the association of birth weight

with raised blood pressure in this group. In young adults born SGA, direct recording of sympathetic nerve traffic using microneurography demonstrates increased sympathetic nerve activity (32), independent of metabolic factors including central adiposity, BMI and glucose tolerance. It has thus been proposed that augmented sympathetic activity may manifest during early development, in those with impaired fetal growth, as a plausible mechanism underlying the observed increased risk of hypertension (32, 33).

However, not all studies have consistently found increases in sympathetic nervous system activity in people born small (Table 1). A study measuring muscle sympathetic nerve activity during rest and in response to a cold pressor test in people born with a low birth weight aged 20-30 years found they had lower sympathetic nerve activity (34). Furthermore, blood pressure and heart rate were similar between low birth weight and healthy birth weight subjects, with no evidence of endocrine differences between the two groups. The functional consequence of the decreased muscle sympathetic nerve activity in low birth weight subjects remains unclear and it is unlikely that the altered sympathetic outflow to the muscle beds is a contributing factor for the higher prevalence of hypertension seen in low birth weight subjects. However, other branches of the sympathetic nervous system, such as the renal or cardiac branch, which control blood pressure, cannot be excluded as these branches may indeed exhibit higher activity.

In addition, other studies have found changes to the autonomic nervous system without an increase or decrease in the sympathetic or parasympathetic branch but showing some autonomic dysfunction. A study assessing heart rate variability a marker of cardiac autonomic control found lower heart rate variability, depicting

diminished cardiac autonomic control in IUGR children (35). Similarly, a study by Rakow and colleagues (2013) showed decreased heart rate variability in 9 year children who were small for gestational age (36).

Taken together, the evidence indicates that people born with impaired fetal growth show altered autonomic activity, putatively predisposing these people to the development of hypertension and CVD in later life.

Evidence for altered autonomic function in individuals born preterm

As early as the first week postnatal, there is evidence that cardiac parasympathetic activity is lower in preterm infants, without alteration in cardiac sympathetic activity (37, 38). At 5-6 months postnatal, both cardiac sympathetic and parasympathetic activity are lower in preterm infants during quiet sleep, but not during active sleep (39), with evidence of reduced sympathetic vasomotor activity in the same infants at 2-4 weeks. Consistent with this observation other studies have also shown, preterm infants exhibit reduced vascular autonomic control, quantified by a reduced baroreflex sensitivity (40) whereby prematurity impairs the normal maturational increase in barorefelx sensitivity (41). The baroreflex is the most important nervous system regulatory mechanism of blood pressure homeostasis. Impaired sensitivity of the baroreflex in these individuals at an early age may place them at an increased risk of cardiovascular instability. Studies investigating autonomic stress responses using postural change test found increased sympathetic response to a head-up tilt test in preterm, growth restricted infants (42, 43). It is possible that the hypertensive phenotype of raised sympathetic activity is only unveiled when infants are challenged with a physiological stressor. Whether such effects persist into childhood and later life is unknown.

Impaired fetal growth is an important risk factor for preterm birth; yet with potentially opposing effects on autonomic activity. Studies investigating prematurity per se found, day old infants born preterm with fetal growth restriction showed reduced cardiac autonomic activity as well as reduced cardiac sympathetic activity compared to their preterm appropriate for age counterparts. These differences seen at postnatal day one did not persist into infancy 1-6 months later. A similar study in 9-year-old children by the same authors found preterm appropriate for gestational age children showed increased parasympathetic activation and blood pressure changes related to respiration compared to term appropriate for gestational age counterparts. It appears these children have a heightened parasympathetic response to respiratory related blood pressure fluctuations, however this is counterintuitive as increase parasympathetic activation is known to be protective of cardiovascular disease (44) and diminished parasympathetic control is known to increase the risk of malignant cardiac arrhythmias and hypertension (38). Other studies in children of similar age found an overall reduction in cardiac autonomic function (36), the overall suppression of cardiac autonomic function may be indicative of an impaired capacity of this group to adapt to changes in the internal and external environment which may contribute to increase risk of later hypertension and cardiovascular disease.

ANIMAL MODELS OF PROGRAMMED HYPERTENSION

Following the strong associations made between low birth weight and increased cardiovascular risk, a number of animal models of programmed hypertension have been studies in the hope of understand the pathophysiological mechanisms. Rat pups with low birth weight show significantly higher blood pressure as adults compared to their normal birth weight litter mates (7), illustrating a consistent neonatal phenotype with that of humans. A number of in utero insults have been found to reduce birth weight and program hypertension in animal models; maternal protein restriction, global food restriction, placental insufficiency and hypoxia, to name a few (45).

Animal models examining the effects of prenatal undernutrition on offspring autonomic nervous system function has found altered autonomic function with the resetting of the baroreflex to a higher set point, increase epinephrine levels, raise catecholamine levels, increase sympathetic and decrease parasympathetic nervous system activity (46). Similarly studies replicating models of IUGR by placental insufficiency accompanied by adult cardiovascular disease has shown that bilateral renal denervation completely abolished established hypertension in the adult offspring (47). This suggests that increased renal sympathetic nerve activity contributes to the maintenance of established hypertension in this model of IUGR (47). A study by Ojeda and colleagues (2007) (48) highlighted that early renal denervation in juvenile rats (4 weeks of age) normalized arterial pressure in IUGR offspring. Furthermore, renal noradrenalin content was increased in IUGR offspring compared to controls (48). Similarly, a study by Jansson and Lambert (1999) (49), found increased sympathetic nerve activity with no changes to blood pressure in IUGR offspring. It is possible that these rats may represent a pre-hypertensive state, therefore studies in the

older rat is required to determine if these rats develop hypertension. Accordingly, there is good experimental evidence to support altered autonomic activity seen in growth restricted individuals.

The exact mechanisms that link impaired fetal growth with changes in autonomic nervous system activity remain poorly described. It is unlikely that low birth weight is the cause of adverse subsequent health outcomes, but simply a surrogate observation. Instead growth restriction reflects systemic restriction of growth including adaptations to counteract the stress by altering structure and function of physiological systems related to blood pressure control. Increased sympathetic activity, higher peripheral vascular resistance, and lower coronary and cerebral arterial resistance are believed to initiate these adaptive changes. Whilst these adaptive changes are important for short-term survival of the growth restricted fetus, these changes may subsequently raise long term risk of cardio-metabolic disease.

Collectively a number of human and animal models demonstrate altered autonomic function following fetal growth restriction and or prematurity. In the context of human studies this is seen throughout the lifespan from infancy to adulthood, (Table 1). However, to date, it is unclear which branch of the autonomic nervous system is increase or decrease. These differences described may be attributed to different methodology employed to measure autonomic control over specific tissues as well as heterogeneity between study populations and participant characteristics. Furthermore, many studies detailing autonomic activity in small babies have been unable to specifically differentiate between the effects of growth restriction, constitutional smallness, and prematurity. Furthermore, birth weight is a widely used, albeit

relatively crude, surrogate of fetal growth. Birth weight percentiles are unable to differentiate between the constitutionally small but well-nourished infant who has met their genetic growth potential, from an under-nourished infant of the same weight, whose intrauterine environment has restricted their growth trajectory. Therefore, defining alterations in fetal growth is problematic leading to heterogeneity within and between study populations. Future studies should take this into consideration. Furthermore, methodological differences between studies may also contribute to the discrepancies seen.

Indeed, preterm birth, constitutional smallness, and fetal growth restriction appear to have distinct associations with later hemodynamic and vascular health (27, 28, 50). These differences may relate to the distinct etiologies, with implications for human populations in developing countries, where maternal malnutrition is an important contributor to fetal growth restriction, and in more developed nations where placental insufficiency is a relatively more important risk factor for impaired fetal growth.

CLINICAL IMPLICATIONS

Hypertension is a complex condition, with multiple risk factors and pathophysiological pathways involved. The concept of developmentally programmed hypertension further our understanding of this disease, yet adds to the complexity. Infants born growth restricted are among the tiniest and most vulnerable babies, and medical advances in the last decades have enabled the first generation of these babies to reach adulthood, but leaving them with an increased risk of hypertension and later cardiovascular disease.

Countering a component of hypertensive disorders that has its origins in utero will open the door to the development of new strategies that prevent or specifically target

these mechanisms. Nonetheless, the exact mechanisms by which in utero exposures alter autonomic activity and subsequent blood pressure remain relatively poorly described. The remarkable similarity of hemodynamic phenotype in offspring exposed to these distinct exposures suggests the possibility that there may be an overarching signal influencing programming in these studies.

In light of the growing number of hypertensive patients it is important to consider a neurogenic origin of hypertension and its exact mechanism, which may enable the development of novel anti-hypertensive agents, which entail more specific targets. Furthermore, low birth weight is a known risk factor for later hypertension with evidence for altered autonomic control. However, currently, there are no clinically established strategies to prevent cardiovascular events in those born growth restricted or preterm. Future studies should look to establish effective pharmacological and nutritional approaches in the management of blood pressure in those born growth restricted or preterm. **Appendix two** contains a published book chapter evaluating a nutritional approach, omega-3 polyunsaturated fatty acid and offspring blood pressure.

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CONFLICT OF INTEREST

None

Table 1. Autonomic cardiovascular control in infants, children and adults born preterm, SGA or IUGR

Reference	Participants	GA (weeks) at birth	PNA at investigation and duration of recording	HR	BP	Overall heart rate variability	Sympathovagal balance	Vascular autonomic control	Response to stressors
Preterm vs term									
Patural *et al.* 2004	23 preterm 8 term *All BW AGA	28 to 37 >37	PT: Theoretical full-term age (38 – 41) Term: 1st wk of life 5 minutes during quiet sleep	N/A	N/A	=	↓ parasympathetic	N/A	N/A
IJzerman *et al.* 2003	32 dizygotic twin pairs 37 monozygotic twin pairs 21 dizygotic twin pairs 24 monozygotic twin pairs	>37 >37 <37 <37	17.0 ± 1.7 y 16.0 ± 1.8 y pre-ejection period RSA	=	↑	N/A	↑ sympathetic = parasympathetic	N/A	↑ sympathetic at rest, reacting time task and during mental arithmetic
Yiallourou *et al.* 2013	25 preterm; BW (600 – 2061g) 31 term; BW (2900 – 4250g)	28 – 32 38 – 42	2 – 4 weeks, 2 – 3 mo, 5 – 6 mo 5 – 8 min (QS & AS)	N/A	N/A	↓	↓ sympathetic ↓ parasympathetic *at 5 – 6 mo (QS)	↑ BPV (HF) ↓ BPV (LF) *(2 – 4 w)	N/A
Andriessen *et al.* 2005	16 Preterm G1; BW (1333 ± 277 g) 10 Preterm G2; BW (1872 ± 468 g) 6 Full term; BW (3220 ± 565 g)	29.3 ± 1.4 32.9 ± 1.2 38.4 ± 1.7	4.7 ± 2.7 d 3.3 ± 1.5 d 3.8 ± 2.1 d 192-s-long segments	N/A	N/A	↓	↓ parasympathetic	↓ Total BPV ↓ BPV (HF)	N/A

Mathewson *et al.* 2014	30 ELBW; BW <1000 g 47 NBW; BW ≥ 2500 g	27.9 ± 2.4 >37	•23.0 ± 1.3 y ELBW, 23.5 ± 1.0 y NBW at (T1) duration 2min. •31.8 ± 1.6 y ELBW, 32.3 ± 1.4 y NBW, (T2); duration 6min	N/A	N/A	= (heart period)	↓ respiratory sinus arrhythmia	N/A	N/A
Patural *et al.* 2007	39 preterm; BW (504-1750 g) 19 full term; BW (2680 – 3640 g)	28 ± 2 40 ± 2	0-14 days 0-7 days 15 min recordings	↑	N/A	↓	=	N/A	N/A
Van Reempts *et al.* 1997	21 preterm + CIUSTR; BW (720-2113 g) 30 preterm control; BW (813-2380 g)	32.8 30.9	2-126 days 2-86 days 15s, 30s and at 1,2,3,4,5 min	N/A	N/A	N/A	N/A	N/A	*Tilt test:* ↑ HR
Schäffer et al. 2008	27 IUGR <5th percentile 27 AGA 10th to 90th percentile	34.3-41.7 34.3-42	PNA not given 24h Holter recording	=	N/A	=	=	N/A	N/A
Term									
Boguszewski *et al.* 2004	12 term AGA 9 term SGA normal stature 8 SGA short stature *Term SGA (BW below -2 SD score for healthy newborns)	> 37	20-25 y postganglionic MSNA	=	=	N/A	↑ sympathetic	N/A	N/A

Galland, *et al*. 2006	27 SGA <10th percentile 23 AGA >25th percentile	39 ± 1 40 ± 1	1 and 3 mo, daytime sleep study, duration 4 h	=	N/A	=	↑ sympathetic ↓ parasympathetic	N/A	Reduced tachycardiac response to head-up-tilt
Weitz *et al.* 2003	13 Term LBW; BW (<2500g) 13 Term NBW; BW (3200-3700)	>37	20 – 30y postganglionic MSNA	=	=	N/A	↓ sympathetic	N/A	= sympathetic response in response to inspiratory apnea & cold pressor test
Aziz *et al.* 2011	33 normal; BW (3530 ± 0.48g) 20 IUGR-1; BW (2290 ± 0.19g) 17 IUGR-2; BW (2290 ± 0.19g)	39.15 ± 1 38. 95 ± 1	8.96 ± 0.72 y 9.31 ± 0.62 y 24h ECG recordings	N/A	N/A	↓ normal vs IUGR-1 = normal vs IUGR-2	=	N/A	N/A
Spassov *et al.* 1994	10 IUGR <3rd percentile 16 AGA 10th to 75th percentile	GA not given conceptional age at study 37-41)	2-10 days 4 hours	↑	N/A	↓	↓ parasympathetic	N/A	N/A
Mixed preterm and term									
Rakow *et al.* 2013	31 preterm; BW (965 ± 202 g) 27 term SGA; BW (2441 ± 334 g) 28 terms; BW (3503 ± 515 g)	26.7 ± 2.1 39.3 ± 1.4 39.6 ± 1.0	9.6 ± 0.3 y 9.8 ± 0.3 y 9.8 ± 0.2 y 24h ECG recordings	N/A	N/A	↓ normal vs Preterm ↓ normal vs SGA	=	N/A	N/A
Cohen *et al.* 2017	25 preterm FGR 22 preterm 19 term AGA *n changed during each follow-up	24-35 25-35 37-41	Day 1, 1mo, 6mo Day 1, 1mo, 6mo N/A, 1mo, 6mo	↑ PT FGR vs PT AGA at 1day during AS &QS	= across 3 groups at 1mo and 6mo	↓ PT FGR vs PT AGA at 1day during AS & QS	↓ PT FGR vs PT AGA at 1day during AS & QS	= BRS across groups during AS & QS	N/A

Yiallourou *et al.* 2017	18 preterm, FGR 15 preterm, AGA 20 term, AGA	30 ± 1 30 ± 1 40 ± 0	9 ± 0.5 y 9 ± 0.5 y 9 ± 0.5 y 2min epochs from overnight recording in AS and QS	=	=	N/A	↑ parasympathetic (PT AGA vs term)	↑ BPV (HF) (PT AGA vs term)	N/A
Souza *et al.* 2017	100 children Prematurity (%) :10 PT, 90 Term	Not specified	8.85 ± 1.82 y	Positively correlated with BMI	Positively correlated with BMI Inverse correlation with BW	N/A	↓ parasympathetic	N/A	N/A
Cohen *et al.* 2008	16 preterm-IUGR BW <10th percentile 16 preterm AGA 29 term AGA	32 ± 2 31 ± 2 40 ± 1	0-2 weeks post-term age	↑preterm (IUGR and AGA) vs term	↓ preterm (IUGR and AGA) vs term	N/A	N/A	N/A	↑ sympathetic response to tilt test • Preterm (IUGR and AGA) vs term; • preterm IUGR vs preterm AGA

GA, gestational age; PNA, postnatal age; HR, heart rate; BP, blood pressure; BW, birth weight; SGA, small for gestational age; AGA, appropriate for gestational age; IUGR, intrauterine growth restricted; CIUST, chronic intrauterine stress; ELBW, extremely low birth weight; LBW, low birth weight; NBW, normal birth weight; FGR, fetal growth restricted; PT, preterm; N/A, not available; MSNA, muscle sympathetic nerve activity; QS, quiet sleep; AS, active sleep; BMI, body mass index; BPV (HF), high frequency component of blood pressure variability; LF:HF, low frequency to high frequency ratio;

↑ increase or ↓ decrease between specified groups, = no differences between specified groups

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CHAPTER THREE

INFLUENCE OF MATERNAL AND PLACENTAL FACTORS ON NEWBORN BODY COMPOSITION

Original Article

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ABSTRACT

Aim: The objective of this study was to assess whether maternal characteristics, placental size or histological chorioamnionitis was associated with newborn body composition. Furthermore, we sought to determine whether placental weight may mediate the association of maternal pre-pregnancy weight and age with newborn body composition.

Methods: A cross sectional study was conducted at Royal Prince Alfred Hospital, Sydney, Australia. This study included 136 healthy singleton term born newborns. Recruitment was stratified by newborn body fat percentiles (sex and gestational adjusted). Body fat was assessed by air displacement plethysmography. A maternal (chorioamnionitis) and fetal (chorionic and umbilical vasculitis, funisitis) inflammatory response were classified according to Redline criteria.

Results: Maternal pre-pregnancy weight, parity, labour, placental weight and surface area were associated with newborn fat mass and fat free mass. Gestational diabetes and maternal age was associated with newborn fat mass but not fat free mass. There was no association between histological chorioamnionitis and newborn body composition however spontaneous onset of labour was strongly associated with the presence of histological chorioamnionitis. Only 25 to 31% of the association of maternal weight and age with newborn fat mass was mediated via the placenta.

Conclusions: Maternal factors associated with newborn fat mass and fat free mass differed, indicating that different mechanisms control fat mass and fat free mass. Our mediation analysis suggests that placental weight partly mediates the association of maternal factors

with newborn body composition. Histological chorioamnionitis was not associated with newborn body composition.

Key words: placenta, chorioamnionitis, newborn body composition

INTRODUCTION

The in-utero environment has a profound influence on fetal development. Sub-optimal in-utero conditions may permanently alter the fetal tissues and organs, changing structure and/or physiological function, and consequently predisposing individuals to later cardiovascular disease (1). A large body of epidemiological evidence supports an association between low birth weight and adult cardiovascular disease (2-6). However, birth weight, is a relatively crude marker of fetal growth and nutrition and does not discriminate well between "normal" small babies and "pathologically" small babies who have failed to meet their growth potential. The same issues arise with defining normally large babies from those who have excessive growth above their expected growth trajectory, also a population at later risk of cardiovascular disease. Measurement of newborn body composition, comprising lean and fat mass, may be a better discriminator of "pathologically" small or large babies. Indeed, measures of fat mass appear to be more associated with the intrauterine environment and maternal nutritional status and correlate better with neonatal morbidity (7). Lean body mass on the other hand is less variable and largely reflects genetic components such as parental size.

Fetal growth is determined by a number of factors, including growth potential related to genetic factors and maternal factors such as stature, nutrition and metabolism, endocrine factors, placental perfusion and function, and the fetal response to growth factors and nutrients (8). Placental function, which regulates nutrient supply to the fetus, is a key determinant of fetal growth and can be modified by both maternal and fetal factors (9). Nutrient transfer is dependent upon the available surface area and thus placental size. Placental weight is used as a crude marker of placental size and is correlated closely with birth weight (10). Studies have found placental weight and the placental weight to birth

weight ratio to be predictive of maternal disease, perinatal morbidity and mortality, as well as childhood growth and development (11-15). Moreover, placental size and placental weight ratio are associated with adult chronic disease outcomes including diabetes, hypertension, coronary artery disease and cerebrovascular incidents (12, 13).

Chorioamnionitis is a perinatal inflammatory exposure most commonly as a response to an ascending intrauterine infection. This affects the chorionic plate and fetal membranes, triggering a maternal inflammatory response. Severe cases of chorioamnionitis can elicit a fetal inflammatory response affecting the umbilical vessels (16). Studies show chorioamnionitis and evidence of persisting systemic inflammation (17, 18). An early life inflammatory event may prime the innate immune system and elicit a heightened inflammatory response to future insults. Inflammation plays a central role in the development of cardiovascular disease, and those born small or large for gestational age show increased risk (2). Therefore, it is possible that a perinatal inflammatory event may contributing to pathways underpinning epidemiological associations between poor intra-uterine growth and adult cardiovascular disease.

Accordingly, we aim to assess whether maternal characteristics, placental size or placental inflammation is associated with newborn body composition. Furthermore, we sought to determine whether placental weight may mediate the association of maternal pre-pregnancy weight and age with newborn body composition.

MATERIALS AND METHODS

Ethical Approval

This study was conducted in accordance with the standards set by the 2013 version of the Declaration of Helsinki and was approved by the Sydney Local Health District ethics committee and The University of Sydney Human Ethics committee (HRECH/14RPAH/478). Participation was voluntary, and informed written consent was collected from the parents of the newborn.

Participants

This is a sub-study of a larger cross-sectional study investigating newborn body composition and markers of cardiovascular risk. We recruited a total of 189 full term newborns on the basis of their body fat percentage from the postnatal wards and Neonatal Unit at Royal Prince Alfred Hospital, Sydney between April 2015 and September 2017 as part of a larger study. Eligible subjects were well singleton newborns between 37 and 42 completed weeks gestation who had body composition measured within 24 hours of birth. Exclusion criteria were major congenital abnormalities and newborns on respiratory support. Of the 189 full term newborns recruited, placental reports were available in 136 full term newborns and were categorised according to established body fat percentiles (19).

Gestational age was calculated from first trimester ultrasounds or date of last menstrual period if no early ultrasound was performed.

Body composition and anthropometry

Newborn body composition was assessed by air displacement plethysmography using the PEAPOD (COSMED, Concord, CA, USA). Air displacement plethysmography is a non-invasive, validated technique for assessing body composition in newborns (20) and utilises

whole body densitometry by accurately measuring the mass and volume of the newborn by calculating fat mass, fat free mass and percentage body fat using software integral to the PEAPOD. Newborn weight was measured by the integrated electronic scales within the PEAPOD measuring to the nearest 0.1g. Newborn volume was calculated by applying Boyles law, relating pressure changes to a given change in volume of air.

Data collection

Maternal and newborn characteristics were collected directly from the mothers and electronic medical records. Maternal body mass index (BMI) was calculated using pre-pregnancy weight and height. Parity was coded as P1 for first pregnancy >20 weeks' gestation and P2+ for those with one or more pregnancies >20 weeks' gestation. Newborn birthweight, head circumference and length and were measured as per the Royal Prince Alfred Hospital Neonatal Early Assessment Program (21).

Placental collection and examination

Placentas that were not required to be sent for formal pathological examination and those not taken home were stored in a 4°C temperature-controlled fridge for 48 hours. After recruitment and consent, placentas were sent to pathology. Placental examination was performed according to the Perinatal Society of Australia and New Zealand Guidelines by an Anatomical Pathologist at the Tissue Pathology and Diagnostic Oncology Department of Royal Prince Alfred Hospital. In our cohort, 74 placentas were sent for placental histological examination according to New South Wales Policy documents detailing Maternity Indications for placental histological examination at time of birth (22). The other 62 placentas were sent to pathology as per study protocol (22).

A Maternal (chorioamnionitis) and fetal (chorionic and umbilical vasculitis, funisitis) inflammatory responses (23) were classified according to Redline criteria for stage (between 1-3) and grade (1 or 2) (16). In our cohort 55 placentas had a reported Redline stage and grade which was reviewed by an anatomical pathologist, 81 of the placental reports were given a Redline stage and grade by a researcher using the same standardised diagnostic terminology for Redline stage and grade (16). Of these, 20 reports were cross-checked further by a neonatal clinician experienced with placental reporting and review and no changes were made.

Placental weight and placental weight to birth weight ratio

Placental weights were measured at pathology with fetal membranes trimmed according to the Amsterdam guidelines (24). Placental weight to birth weight ratio was calculated by dividing the placental weight (g) by birth weight (g).

Placental surface area

Assuming an elliptical surface area, placental surface area was calculated as $maximal \times lesser\ diameter \times \pi/4$ (25).

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences, IBM Corp, version 23). Data expressed as mean and standard deviations. Associations of maternal and placental factors associated with newborn fat mass were determined by Pearson's correlation and multivariable linear regression for continuous variables and by logistic regression for categorical variables. Each variable in the multivariable models was adjusted for newborn sex and gestational age.

We used a simple mediation model derived from multivariable linear regression to determine whether placental weight may mediate the association of maternal factors (particularly maternal pre-pregnancy weight and age) with newborn fat mass (26). Separate mediation models were conducted for each maternal factor. Beta coefficients and 95% confidence intervals were calculated for all models. The 95% confidence intervals for the indirect effect were calculated using the bootstrapping method with 10,000 replications. The mediation analysis was performed by the SPSS macro PROCESS (26). Percent mediation (P_M) was calculated as the percent of the total effect accounted for by the indirect effect.

RESULTS

Participants

Placental reports were available in 136 full term newborns across body fat percentiles; $\leq 10^{th}$ (n = 25), >10th to $\leq 25^{th}$ (n = 21), >25th to $\leq 50^{th}$ (n = 24), >50th to $\leq 75^{th}$ (n = 17), >75th to $\leq 90^{th}$ (n = 22), $>90^{th}$ (n = 27) (Table 1). The 74 placentas sent up front did not have significant placental abnormalities and therefore has not affected outliers.

Factors associated with newborn fat mass and fat free mass

Maternal factors associated with newborn fat mass in unadjusted analysis were pre-pregnancy weight (r = 0.227, P = 0.01), maternal age (r = 0.211, P = 0.01) and parity (r = 0.178, P = 0.02). These effects remained similar when adjusted for sex and gestational age of the newborn. Gestational diabetes mellitus and type of labour were also associated after adjustment (Table 2). In our unadjusted analysis, only pre-pregnancy weight was associated with newborn fat free mass (r = 0.241, P = 0.003). These effects remained similar when adjusted for sex and gestational age with parity and labour also being associated after adjustment (Table 2).

Placental factors associated with newborn fat mass in unadjusted analyses were, placental weight ($r = 0.472$, $P < 0.0001$) and placental surface area ($r = 0.263$, $P = 0.01$). Similarly, placental weight and placental surface area were both associated with newborn fat free mass ($r = 0.323$, $P < 0.0001$), ($r = 0.225$, $P = 0.004$). These remained similar when adjusted for sex and gestational age (Table 2).

Maternal factors associated with placental weight and histological chorioamnionitis

In our unadjusted analyses only maternal age was associated with placental surface area ($r = 0.147$, $P = 0.04$), however, after adjusting for newborn sex and gestational age this association was no longer significant (Table 3). Parity was associated with the presence of chorioamnionitis and a fetal inflammatory response, furthermore spontaneous onset of labour was associated with chorioamnionitis. These remained similar after adjustment for sex and gestational age (Table 4).

Mediation analysis

A mediation analysis was performed to determine whether placental weight, may account for a proportion of the shared variance between selected maternal factors and newborn fat mass. Figure 1 provides a visual representation of the mediation model. Maternal factors included in the mediation model were maternal pre-pregnancy weight and maternal age and are displayed in (Figure 1.)

This analysis revealed that the association of maternal pre-pregnancy weight and maternal age with newborn fat mass did not appear to be solely mediated by placental weight, (Figure 1). The mediator (placental weight) accounted for $P_M = 0.31$ and $P_M = 0.26$ percent of the

total effect of maternal pre-pregnancy weight and maternal age with newborn fat free mass, although these indirect effects did not reach statistical significance, (Figure 1).

DISCUSSION

The placenta is vital in normal fetal growth in utero. Most studies assessing placental structure or function assess the relationship with birth weight. Birth weight however is a relatively crude marker of baby's morbidity risk and in recent years various methods of assessing body composition appear to correlate better (7). There are few studies assessing placental factors and newborn body composition. We found, placental weight and surface area, to be associated with both newborn fat mass and fat free mass. Our mediation analysis indicates that an estimated 25 to 31% of the associations of maternal weight and age with newborn fat mass are mediated via placental weight. This study found no evidence for an association between chorioamnionitis with newborn fat mass or fat free mass.

Maternal pre-pregnancy weight, age, parity, gestational diabetes and labour were positively associated with newborn fat mass. Conversely, newborn fat free mass was associated with maternal pre-pregnancy weight, parity and type of labour but not gestational diabetes or age. Newborn fat mass may be a better indicator of energy supply and growth of the fetus than birth weight as increased neonatal fat may be found in normal weight neonates. Furthermore, neonatal fat mass is thought to better reflect the intrauterine environment and energy supply in the last trimester better whereas lean mass is more reflective of genetic influences (27). The differentiated effects may suggest that the mechanisms controlling fat mass and fat free mass are different.

Maternal factors may affect fetal growth by modifying placental nutritional transport. The transport capacity depends on total surface area as well as the efficacy of transporters. In our study both placental weight and surface area was associated with newborn fat mass, table 2. We hypothesised that the association of selected maternal factors with newborn fat mass may be mediated via the placenta, specifically, placental weight. This hypothesis was somewhat supported by our mediation analysis. Although the indirect pathway via placental weight did not reach statistical significance, we suspect this is primarily due to limited statistical power. It possible that other mechanisms that we were unable to assess may be involved. Future studies with a larger sample size would quantify the strength of these mediation pathways, and also to expand such pathways to include maternal total weight gain during pregnancy.

There is increasing evidence that an early life inflammatory exposure may prime the innate immune system, subsequently resulting in a heightened inflammatory response in the future (17). This may contribute to pathways underpinning epidemiological associations between poor intra-uterine growth and increased cardiovascular risk. We found no evidence for an association between chorioamnionitis and newborn body composition. However, in line with previous studies, spontaneous onset of labour was associated with chorioamnionitis, furthermore spontaneous onset of labour was positively associated with newborn fat mass and fat free mass. Longitudinal studies investigating infants exposed to chorioamnionitis is need. Larger studies should also look to exclude laboured placentas as these placentas may appear to be more.

We acknowledge the limitations of this study, including a relatively small sample size and restricted assessment of maternal factors that may influence the placental. Subsequent research in this field should include measurement of gestational weight gain and maternal

body composition. However, to our knowledge this is the first study to assess maternal factors, placental size and inflammation and newborn fat mass and the first to attempt to delineate direct and indirect associations. Future studies should address these limitations in terms of breadth of data and sample size, in order to better elucidate the causal pathways that underpin the observed associations and inform further on the putative mechanisms linking perinatal exposures with later cardio-metabolic risk.

In conclusion, this study found that maternal factors associated with newborn fat mass and fat free mass differed. These differentiated effects suggest that the mechanisms controlling fat mass and fat free mass may be different. Placental weight and surface area was strongly associated with newborn fat mass and fat free mass. Interestingly only 25 – 31% of the effect of maternal pre-pregnancy weight and maternal age on newborn fat mass was mediated by placental weight. This study found no evidence for an association between chorioamnionitis and newborn body composition.

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CONFLICT OF INTEREST

None

Table 1: Participant characteristics

Maternal characteristic n = 136	Value
Age, y	33.7 (3.9)
Ethnicity, n (%)	
Asian	34 (25)
Caucasian	89 (65)
Middle Eastern	4 (3)
Other	8 (6)
Not stated	1 (1)
Maternal smoking, n (%)	4 (3)
Maternal pre-pregnancy BMI, (Kg/m^2)	23.7 (4.4)
Maternal pre-pregnancy weight, (kg)	63.7 (12.8)
Gestational diabetes mellitus, n (%)	22 (16.2)
Hypertension, n (%)	4 (2.9)
OGTT 2 hours, (mmol/L)	5.9 (1.5)
Parity, n (%)	
P1	85 (63)
P2+	51 (38)
Mode of delivery, n (%)	
Normal	76 (55.9)
Instrumental	25 (18.4)
Caesarean	35 (25.7)
Labour, n (%)	
Spontaneous	71 (52.2)
Induced	42 (30.9)
No labour	23 (16.9)
Newborn characteristics n = 136	**Value**
Gestational age, (weeks)	39.2 (1.1)
Female, n (%)	69 (50.7)
Length, (cm)	49.7 (2.3)
Head circumference, (cm)	34.7 (1.5)
Birth weight, (g)	3385 (540)
Body fat, n (%)	10.8 (5.4)
Body fat, (g)	405 (351)
Fat free mass, (g)	89.2 (5.3)
Fat free mass, (g)	2944 (483)

Data presented as mean (SD) for continuous variables and n (%) for dichotomous variables. *N* = 136, except for maternal BMI (n = 124), pre-pregnancy weight (n = 130), and 2 hours oral glucose tolerance test (OGTT) (n = 125).

Table 2: Maternal and placental factors associated with newborn body composition

	Newborn fat mass (g) n = 136		Newborn fat free mass (g) n = 136	
	β (95% CI)	*P* value	β (95% CI)	*P* value
Newborn sex (male)	80 (-36, 196)	0.17	-188. (-347, -30)	0.02
Gestational age (weeks)	73 (21, 124)	0.01	92 (21, 163)	0.01
Maternal Characteristics				
†Maternal BMI (Kg/m^2)	10 (-5, 24)	0.19	8 (-11, 27)	0.40
†Pre-pregnancy weight (kg)	6 (11)	0.01	9 (2, 15)	0.01
†Maternal age (years)	22 (7, 36)	0.004	11 (-9, 32)	0.27
†Parity (P1 vs P2+)	205 (89, 321)	0.01	180 (16, 342)	0.03
†Gestational diabetes mellitus	185 (24, 346)	0.02	-14 (-238, 211)	0.91
†OGTT, 2 hours (mmol/L)	3 (-25, 31)	0.83	-14 (-73, 46)	0.65
Placental Characteristics				
†Placental weight (per 100g)	126 (84, 167)	<0.0001	116 (54, 178)	<0.001
†Placental surface area (cm^2)	1 (0.4, 2)	<0.01	1 (0.4, 3)	0.01
†Placental weight to birth weight ratio	109 (-2089, 2304)	0.92	-2316 (-5285, 653)	0.13
†Villitis	86 (-105, 277)	0.37	180 (-80, 440)	0.17
†Histological chorioamnionitis	-90 (-211, 32)	0.15	-145(-310, 20)	0.08
†Fetal inflammatory response	153 (-33, 339)	0.11	210 (-45, 465)	0.11

Values are unstandardized β-regression coefficients (95% CI) from multiple multivariable models. †Each variable is adjusted for sex and gestational age. Results shown are increase in newborn fat mass (g) and fat free mass in (g) per unit increase in the independent variable. BMI, body mass index; OGTT, oral glucose tolerance test.

Table 3: Maternal factors associated with placental weight and placental surface area

	Placental weight (g) n = 136		Placental surface area (cm^2) n = 136	
	β (95% CI)	*P* value	β (95% CI)	*P* value
Newborn sex (male)	-6 (-51, 38)	0.78	-3 (-30, 23)	0.81
Gestational age (weeks)	13 (-7, 33)	0.21	2 (-10, 14)	0.73
†Maternal BMI (kg/m^2)	1 (-4, 6)	0.66	-1(-4, 2)	0.59
†OGTT, 2 hours (mmol/L)	3 (-12,18)	0.70	4 (-5, 13)	0.41
†Maternal age (years)	5 (-1, 10)	0.10	3(-1, 6)	0.07
†Parity (P1 vs P2+)	40 (-3, 85)	0.07	29 (3, 55)	0.03
†Hypertension of pregnancy	-4 (-130,123)	0.96	-41 (-114, 33)	0.28
†Gestational diabetes	-21 (-82, 39)	0.48	22 (-13, 57)	0.23
†Pre-pregnancy weight (kg)	1.4 (-0.3, 3)	0.10	0.4 (-0.6, 1.4)	0.44

Values are unstandardized β-regression coefficients (95% CI) from multiple multivariable model. † Each variable is adjusted for sex and gestational age. Results shown are increase in placental weight (g) and placental surface area (cm^2) per unit increase in the independent variable. BMI, body mass index; OGTT, oral glucose tolerance test.

Table 4: Maternal factors associated with chorioamnionitis and fetal inflammatory response

	Presence of histological chorioamnionitis n = 49/136		Presence of fetal inflammatory response n = 15/136	
	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Newborn sex (male)	0.86 (0.42, 1.75)	0.68	0.34 (0.10, 1.14)	0.08
Gestational age (weeks)	1.29 (0.93, 1.78)	0.12	0.86 (0.53, 1.38)	0.52
†Maternal BMI (kg/m^2)	0.97 (0.88, 1.06)	0.44	1.08 (0.91, 1.27)	0.38
†Maternal age (years)	0.93 (0.84, 1.02)	0.12	0.97 (0.85, 1.15)	0.68
†Parity (P1 vs P2+)	2.25 (0.11, 0.58)	0.001	9.45 (1.19, 74.91)	0.03
†Hypertension of pregnancy	2.00 (0.26, 15.08)	0.50	0.22 (0.02, 2.51)	0.22
† OGTT, 2 hours (mmol/L)	0.94 (0.73, 1.21)	0.65	1.28 (0.85, 1.95)	0.24
†Gestational diabetes	1.83 (0.61, 5.51)	0.28	---#	
†Spontaneous onset of labour	3.36 (1.57, 7.17)	0.002	0.50 (0.16, 1.60)	0.25
†Maternal pre-pregnancy weight (kg)	0.98 (0.95, 1.01)	0.23	1.04 (0.98, 1.11)	0.20

Values are odds ratios (95% CI) from multiple multivariable model. † Each variable is adjusted for sex and gestational age. # Of the 22 participants with gestational diabetes, there were none with a fetal inflammatory response. BMI, body mass index; OGTT, oral glucose tolerance test.

Figure 1: Mediation models

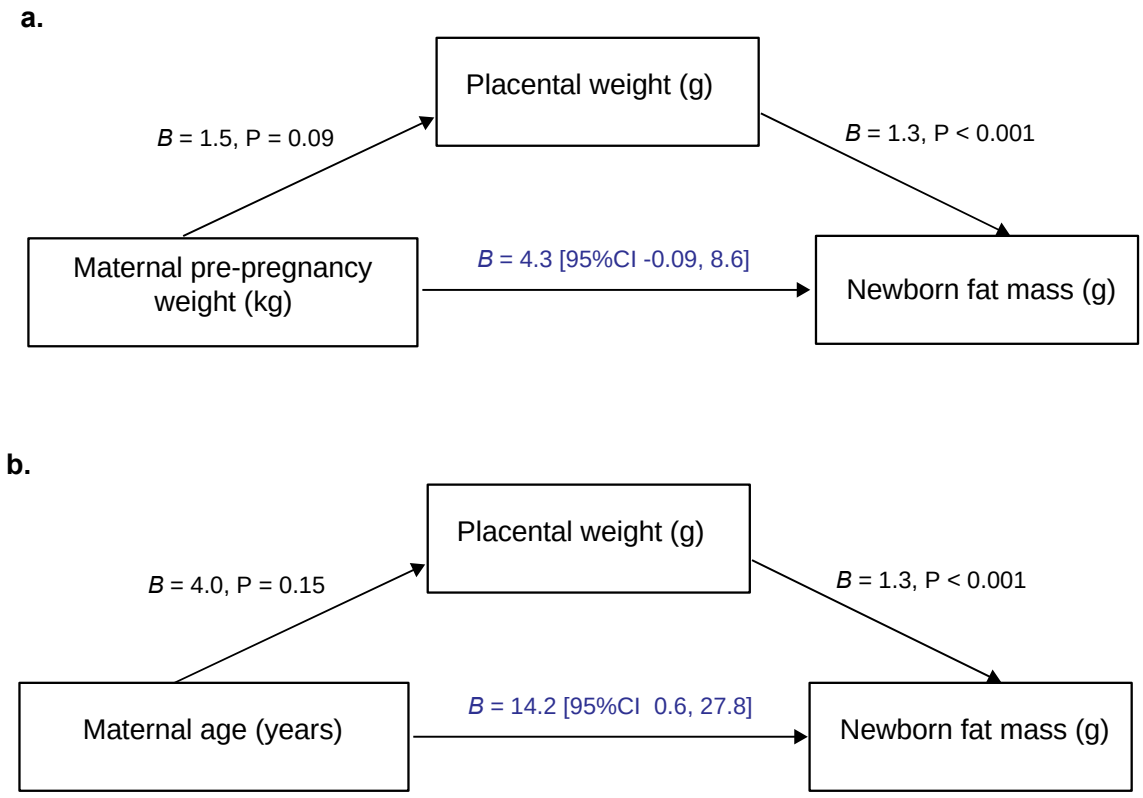

Figure 1: Mediation models showing the effect of maternal factors on newborn fat mass with the mediator, (placental weight) located causally between maternal factors and newborn fat mass.
a) The direct effect of maternal pre-pregnancy weight when controlled for placental weight is indicated in blue. The indirect effect via placental weight was *B* = 1.933 [95%CI -0.01,4.64]. **b)** The direct effect of maternal age when controlled for placental weight is indicated in blue. The indirect effect via placental weight was *B* = 5.058 [95%CI -0.16,12.39].

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CHAPTER FOUR

NONINVASIVE ASSESSMENT OF AUTONOMIC FUNCTION IN HUMAN NEONATES BORN AT THE EXTREMES OF FETAL GROWTH SPECTRUM

Original Article

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ORIGINAL RESEARCH

Noninvasive assessment of autonomic function in human neonates born at the extremes of fetal growth spectrum

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Keywords
Autonomic function, hypertension, in utero growth, newborn body fat.

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Abstract

Birth weight is associated with adult cardiovascular disease, such that those at both ends of the spectrum are at increased risk. This may be driven in part by modification to autonomic control, a mechanistic contributor to hypertension. However, birth weight is a relatively crude surrogate of fetal growth; and newborn body composition may more accurately identify the "at risk" infant. Accordingly, we sought to determine whether newborns with high or low body fat have altered autonomic control of vasomotor function and cardiac contractility. Body fat was assessed by air-displacement plethysmography <24 h postnatal. Measures of spontaneous baroreflex sensitivity (sBRS), blood pressure variability (BPV), and dP/dt_{max} variability were compared between newborns categorized according to established body fat percentiles: high body fat (HBF, >90th percentile, $n = 7$), low body fat (LBF, ≤10th percentile, $n = 12$), and normal body fat (control, >25th to ≤75th percentile, $n = 23$). BPV was similar across body fat percentiles; similarly, low frequency dP/dt_{max} variability was similar across body fat percentiles. sBRS was reduced in HBF compared to controls (11.0 ± 6.0 vs. 20.1 ± 9.4 msec/mmHg, $P = 0.03$), but LBF did not differ (18.4 ± 6.0 msec/mmHg, $P = 0.80$). Across the entire body fat spectrum ($n = 62$), there was a nonlinear association between newborn body fat and sBRS ($P = 0.03$) that was independent of birth weight ($P = 0.04$). Autonomic modulation of vasomotor function and cardiac contractility in the newborn did not differ by body fat, but newborns born with high body fat show depressed baroreflex sensitivity.

Introduction

Hypertension is an important modifiable risk factor for cardiovascular and cerebrovascular diseases. Despite decades of research, over 95% of all cases of hypertension remain of unknown etiology (World Health Organisation, 2013), necessitating that treatments target the established symptoms and not the cause. A major consequence of this is a failure of the treatment strategies currently used in the management of hypertension with a considerable number of patients failing to adequately control their blood pressure (Yaxley and Thambar 2015).

A key pathophysiological hallmark of hypertension is elevated sympathetic nerve activity. Importantly, raised sympathetic activity has been reported in prehypertensive cohorts such as in patients with a family history of hypertension and may even be established during childhood (Julius et al. 1991). Thus, sympathetic overactivity precedes hypertension

and may be a pathophysiological component of the causal pathway. Impaired baroreceptor reflex function has also been reported in both established hypertension (Bristow et al. 1969; Gribbin et al. 1971; Ducher et al. 2006), and in young adults with borderline hypertension (Takeshita et al. 1975; Matsukawa et al. 1991).

One of the most important recent advances in hypertension research is an understanding that hypertension often may have a developmental origin. This concept is based upon epidemiological studies where babies born small had a significantly higher prevalence of hypertension and cardiovascular disease in later life (Lawlor et al. 2002). People born small for gestational age represent a group at one end of the birth weight spectrum who are at increased risk of cardiovascular disease in adulthood, however, more recently those born large for gestational age also represents a group at increased risk (Koklu et al. 2007; Skilton et al. 2014).

Birth weight is unable to differentiate between the constitutionally small but well-nourished newborn who has met their genetic growth potential, from an undernourished newborn of the same weight, whose intrauterine environment has restricted their growth trajectory. Similarly, those who are large for gestational age may be constitutionally large, or have excessive fetal growth above their expected growth trajectory. It has been proposed that groups of newborns with restricted or excessive growth in response to their intrauterine environment are those at most risk of later cardiovascular disease (Barker et al. 1993). Therefore, utilization of newborn body composition, comprising lean and fat mass may be a better indicator than birth weight.

Being able to measure autonomic function noninvasively and early detection of hypertension risk at a young age may help both in understanding the etiology of hypertension and identifying those at risk at a young age, which could prove highly valuable.

Accordingly, we sought to determine whether vascular autonomic function, specifically blood pressure variability and baroreflex function, differ by newborn percentage body fat and prematurity. Furthermore, this study has introduced a possible new noninvasive measure of autonomic regulation of the myocardium, the dP/dt_{max} variability in arterial pressure.

Methods

Ethical approval

This study was conducted in accordance with the standards set by the 2013 version of the *Declaration of Helsinki* and was approved by the Sydney Local Health District ethics committee and The University of Sydney Human Ethics committee (HRECH/14RPAH/478). Participation was voluntary, and informed written consent was collected from the parents of the newborn.

Subject selection

Participants were recruited from the postnatal wards and the neonatal unit at Royal Prince Alfred Hospital, Sydney. Eligible newborns were singleton newborns between 37 and 42 completed weeks of gestation and those born late preterm between 34 and 36 weeks of gestation who had undergone routine body composition measurements. The only exclusion criterion for this study was major congenital abnormalities and ongoing need for respiratory support in the newborn. Obstetric assessment of pregnancies at risk of abnormal fetal growth (such as preeclampsia) was not excluded. Gestational age was calculated from first trimester ultrasounds.

Body composition and anthropometry

Body composition was measured in infants in the first 24 h of life with air-displacement plethysmography (PEA POD®, COSMED USA, Inc), as part of routine clinical practice. Air-displacement plethysmography is an age-appropriate method for assessing body composition (Fields et al. 2015), and has been validated in both term and preterm infants (Ma et al. 2004; Ellis et al. 2007; Roggero et al. 2012). This technique accurately measures body volume by the application of Boyle's law to the displacement of air by the infants in a sealed chamber. Fat mass and fat-free mass are calculated by proprietary algorithms. Anthropometry was measured concurrently by two trained midwives. Weight is measured with the integrated PEA POD® scales to the nearest gram, and head circumference to 0.1 cm. Length is measured with a length board to the nearest 0.1 cm (Easy-Glide Bearing infantometer, Perspective Enterprises, USA). Newborns were categorized according to published body fat percentiles, adjusted for gestational age and gender (Hawkes et al. 2011).

Data collection

Maternal and newborn characteristics were collected directly from mothers using a standardized questionnaire and corroborated from electronic medical records.

Subjects

Blood pressure was recorded in 113 newborns, although the measurements from 43 of these were insufficient for analysis due to newborn- or equipment-related problems. Failed measurements that were newborn-related occurred when the newborn woke up during the measurement,

failed to settle into sleep, or when the measurement was interrupted by a member of the clinical care team. Equipment-related problems occurred when the blood pressure cuff failed to detect sufficient or continuous blood pressure. Adequate blood pressure waveforms were obtained in 70 newborns, of these 8 were late preterm newborns and 62 were full-term newborns categorized according to established body fat percentiles (Hawkes et al. 2011) as follows: ≤10th percentile (n = male/female), n = 7/5; >10th to ≤25th, n = 5/5; >25th to ≤50th, n = 5/8; >50th to ≤75th' n = 5/5; >75th to ≤90th' n = 4/6; >90th percentile, n = 4/3.

To ensure equal spread across body fat percentiles for comparisons between the full-term and late preterm group, the full-term group was selected so that 10% of newborns were ≤10th body fat percentile, 15% from >10th to ≤25th, 25% from >25th to ≤50th, 25% from >50th to ≤75th, 15% from >75th to ≤90th and 10% of newborns >90th percentile body fat percentile. Individuals within body fat percentiles were chosen at random.

Data acquisition

Continuous blood pressure recordings were acquired in the sleeping newborn at 1–5 days old using a noninvasive photoplethysomgraphic cuff (Finometer Pro, FMS, Finapress Medical Systems, The Netherlands), placed around the right wrist of the newborn with the sensor positioned over the radial artery. Blood pressure was recorded in 4-minute intervals and repeated 1–3 times in each newborn. Analogue outputs of blood pressure were sampled at 500 Hz using Labchart program (ADInstruments, Sydney, Australia). Continuous blood pressure recordings were exported to Spike2 software (version 7.18, Cambridge Electronics Design, Cambridge, UK) and the following waveforms were generated: systolic blood pressure (SBP), dP/dt, and dP/dt_{max} of the blood pressure waveform.

SBP variability

Power spectral analysis of the systolic blood pressure waveform provides a noninvasive method for the analysis of autonomic nervous system modulation to vasculature. SBPV was calculated using frequency domain methodology on the SBP waveform sampled at 5 Hz, with linear trend removal and by performing a Fast Fourier transform (FFT, 256 point, Hanning window, zero percent overlap) using customized algorithms in Spike2. The FFT was performed on SBP waveform durations of 1- to 2-min epochs repeated 3–5 times and averaged in each infant, methodology similar to those previously published in infants (Yiallourou et al. 2012a, 2013). Spectral bands of SBPV were defined at 0.04–0.15 Hz for low frequency and 0.15–1.1 Hz for high frequency. The very low-frequency band was not analyzed because of the short time period of the recordings. The high-frequency band was based on respiratory rates in newborns at 0.5–1 Hz (Polson et al. 2006) and the total frequency band was defined as the range between 0 and 1.1 Hz.

The low-frequency component of SBP variability is an established biomarker for sympathetic modulation of the vasculature. These low-frequency oscillations of systolic blood pressure are a result from an oscillation of the sympathetic vasomotor tone and are enhanced during sympathetic activation (Julien 2006).

Spectral analysis of dP/dt_{max} variability

Estimated arterial dP/dt_{max} has been reported to be a surrogate measure for evaluating changes in left ventricular contractility (Rhodes et al. 1993), and dP/dt_{max} variability may represent autonomic modulation of ventricular myocardial contractility. The arterial pressure dP/dt is determined by the first differential of the blood pressure waveform, and dP/dt_{max}, the peak rise in blood pressure was identified using a peak detection algorithm in Spike2 (Fig. 1). Frequency analysis of dP/dt_{max} variability was then performed similar to SBPV (Fig. 2). The dP/dt_{max} waveform was resampled at 5 Hz, and linear trend removal and FFT (256 point, Hanning window) was performed. As the maximum rate of change in arterial pressure is related to the force of ventricular contraction, the low- frequency component of dP/dt_{max} variability may reflect the sympathetic modulation of the myocardium during systole.

Measures of low and high power for SBPV and dP/dt_{max} were explored in its absolute values and using normalized units (nu) as some studies indicating power spectral components which are normalized for total power may better detect sympathetic and parasympathetic predominance (Pagani et al. 1997). Normalized units were calculated as previously described (Malik et al. 1996) using the equation below, as an example for normalized units of low frequency:

$$\text{LF(NU)} = (\text{LF}/(\text{Total Power-VLF})) \times 100$$

Baroreflex function analysis

Spontaneous baroreflex sensitivity (sBRS) was determined using the sequence method which incorporates the identification of sequences of consecutive increases in SBP (pressor ramps) or decreases in SBP (depressor ramps) that are followed by a progressive lengthening (or shortening) of pulse interval (PI) (Polson et al. 2006). Spontaneously occurring changes in SBP over a period of four

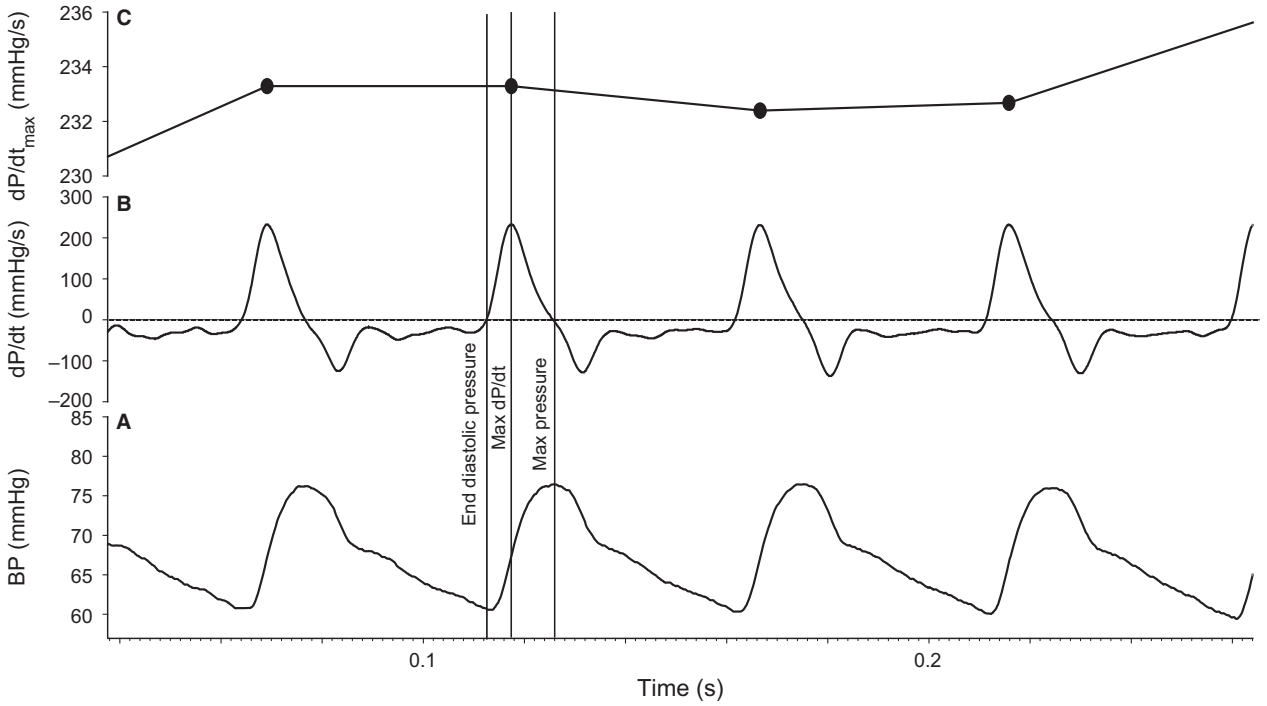

Figure 1. Example of blood pressure waveform (A) recorded in a control newborn using the Finapres and (B) d*P*/d*t* and (C) d*P*/dt$_{max}$ derived from the BP waveform. The dP/dt waveform was generated by applying the slope function to the blood pressure waveform in Spike2. d*P*/dt$_{max}$ coincides with the maximum upstroke of the blood pressure waveform during systole.

or more beats were identified and the relationship with the corresponding pulse interval, with delays of three, four, and five beats were plotted. These delays were chosen based on a delay of approximately 2 sec for changes in heart rate in response to a change in blood pressure, given that the neonatal heart rate is ~2 beats per second (Polson et al. 2006). The slope and r^2 value of the linear regression for these plots were calculated and a baroreceptor mediated change in heart rate was only considered to have occurred when the slope was positive and r^2 was >0.8 for each delay.

In addition to sBRS, baroreflex effectiveness index (BEI) was used as an additional measure of baroreflex function in the newborn. This is a measure of barorflex recruitment and was determined as the ratio of the number of identified baroreflex sequences against the total number of SBP ramps observed for a given period of time (Rienzo et al. 2001), calculated using the formula below:

$$\text{BEI} = \frac{\text{Total number of pulse interval/SBP sequences}}{\text{total number of SBP ramps}}$$

Statistical analysis

Statistical analysis was performed using SPSS Statistics, version 23 (IBM Corp, Armonk, N.Y., USA). Continuous data were expressed as mean (standard deviation), and categorical data as count (percentage). Data were visually inspected for normality, and nonnormally distributed data was transformed appropriately. One-way analysis of variance (ANOVA), with Dunnett correction for multiple comparisons was applied to compare high body fat (HBF; >90th percentile) with control (body fat >25th to ≤75th percentile), and low body fat (LBF; ≤10th percentile) with control newborns. Full-term and late preterm newborns were compared using independent-samples *t*-test. Chi-square tests were used for categorical data. Nonlinear associations were determined by use of quadratic terms in multivariable regression models that included participants across the entire spectrum of body fat percent. Statistical significance was inferred where $P < 0.05$.

Results

Participant characteristics

Of the total 62 full-term newborns, 7 were HBF (>90th body fat percentile), and 12 were LBF (≤10th body fat percentile) and 23 newborns in the control group (>25th to ≤75th body fat percentile). Mothers who gave birth to newborns with HBF had a greater proportion of

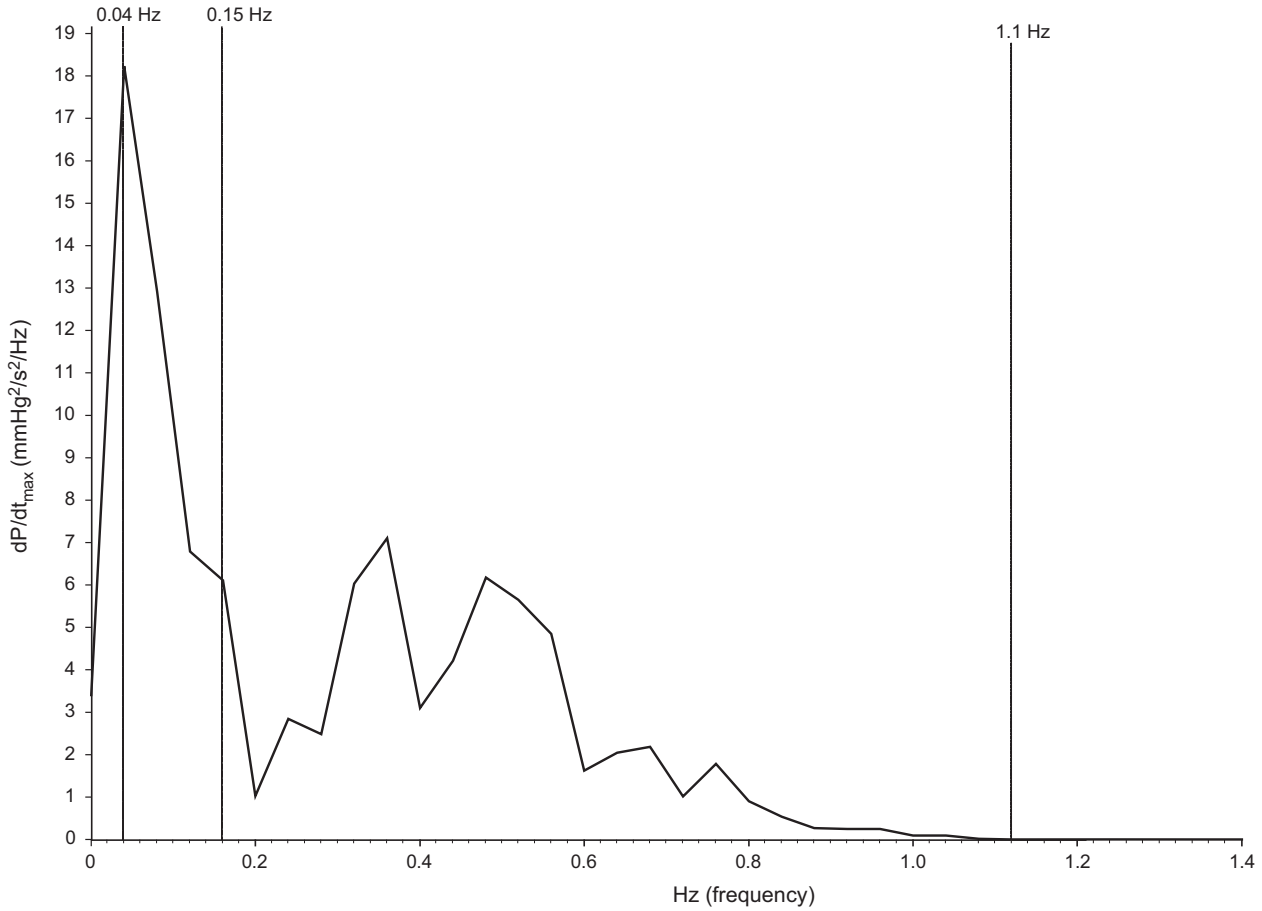

Figure 2. Estimated power spectral density obtained from a 60-sec period of dP/dt_{max}. The *y*-axis represents dP/dt_{max} variability in $mmHg^2/sec^2/Hz$ and the *x*-axis represents frequency in Hz. The bin size was 0.04 Hz. The vertical lines denote the ranges for the very low-frequency band (<0.04 Hz), the low-frequency band (0.04–0.16 Hz), the high-frequency band (0.16–1.1 Hz). Total power is the sum of the area under the curve between 0 and 1.1 Hz.

cesarean deliveries and no labor compared to controls. Mothers in the LBF and control group were more likely to have a spontaneous, normal (vaginal) delivery, (Table 1).

Newborns with LBF were lighter, shorter, and had reduced head circumference compared to controls, while newborns with HBF were heavier, taller, and had a larger head circumference. As expected, based on selection, newborns with LBF had reduced body fat compared to controls, while newborns with HBF had higher body fat. Conversely, LBF newborns had increased fat-free mass compared to controls, while newborns with HBF had reduced fat-free mass, (Table 1).

Maternal characteristics were not different between the full-term and late preterm group. Late preterm newborns were lighter, shorter, had reduced head circumference, body fat, and reduced fat-free mass compared to full-term newborns, (Table 2).

Autonomic function between newborn body fat percentiles

Systolic blood pressure variability

We found no differences in overall blood pressure variability or individual frequency components of blood pressure variability across body fat percentiles. Similarly, the normalized units of low and high frequency components were not different across body fat percentiles, (Table 3).

dP/dt_{max} variability

We found no differences in overall dP/dt_{max}, dP/dt_{max} variability or individual frequency components of dP/dt_{max} variability across body fat percentiles. Similarly, the normalized units of low- and high-frequency components were not different across body fat percentiles, (Table 3).

Table 1. Maternal and newborn characteristics across body fat percentiles.

	LBF ≤ 10th percentile (n = 12)	Control > 25th to ≤75th percentile (n = 23)	HBF > 90th percentile (n = 7)	P value
Maternal characteristics				
Age (years)	32 ± 4.4	34 ± 4.3	36 ± 6.6	0.26
Ethnicity, *n* (%)				
Asian	3 (25)	4 (17)	0 (0)	
Caucasian	4 (33)	15 (65)	5 (71)	
Middle Eastern	1 (8)	0 (0)	0 (0)	0.50
South Asian	3 (25)	3 (13)	1 (14)	
Other	1 (8)	1 (4)	1 (14)	
Maternal prepregnancy BMI (kg/m^2)	21.9 ± 2.6	22.8 ± 3.5	22.8 ± 2.3	0.71
Gestational diabetes mellitus, *n* (%)	2 (17)	5 (22)	1 (14)	0.88
Preeclampsia, *n* (%)	1 (8)	0 (0)	0 (0)	0.28
NICU admissions, *n* (%)	1 (8)	2 (9)	0 (0)	0.71
Glucocorticoid exposure, *n* (%)	0 (0)	1 (4)	0 (0)	0.66
Maternal smoking, *n* (%)	0 (0)	0 (0)	0 (0)	–
Hypertension in pregnancy, *n* (%)	1 (8)	0 (0)	0 (0)	0.28
Mode of delivery, *n* (%)				
Normal delivery	10 (83)	13 (57)	2 (29)	
Instrumental	0 (0)	7 (30)	1 (14)	0.02
Cesarean	2 (17)	3 (13)	4 (57)	
Labor				
Spontaneous	6 (50)	14 (61)	2 (29)	
Induced	5 (35)	8 (35)	1 (14)	0.01
No labor	1 (8)	1 (4)	4 (57)	
Newborn characteristics				
Gestational age (weeks)	38 ± 1.0	39 ± 1.3	39 ± 1.1	0.18
Sex (girls/boys)	5/7	13/10	3/4	0.65
Birth weight (g)	2772 ± 332	3354 ± 469	4210 ± 315	<0.0001
Length (cm)	48 ± 1.8	50 ± 2.5	54 ± 1.7	<0.0001
Head circumference (cm)	33 ± 1.1	35 ± 1.5	36 ± 0.5	<0.0001
Body fatness (%)	3 ± 2	10 ± 2	17 ± 2	<0.0001
Body fatness (g)	98 ± 57	356 ± 101	728 ± 89	<0.0001
Fat-free mass (%)	97 ± 1.8	90 ± 2	83 ± 5	<0.0001
Fat-free mass (g)	2665 ± 302	3010 ± 400	3467 ± 299	<0.0001

Data are presented as mean ± SD for continuous data using one-way analysis of variance (ANOVA), and *n* (%) for categorical data, using chi-square tests between newborn body fat percentiles. LBF; low body fat percentile, HBF; high body fat percentile, NICU; neonatal intensive care unit.

Baroreflex function

Spontaneous baroreflex sensitivity (sBRS) was significantly different between body fat percentiles (P = 0.04, Table 3). Multiple comparisons revealed that sBRS was ~45% lower in newborns with HBF than controls (P = 0.02), however, no differences were seen between LBF and controls.

Across the entire body fat spectrum, there was a non-linear association between infant body fat percent and sBRS (P = 0.03, adjusted for gestational age and sex), which was independent of birth weight (P = 0.04 after adjustment). Body fat percent accounted for 13.4% of the variance in sBRS. In comparison, birth weight accounted for 10% of the variance in sBRS. In a model which included birth weight, the introduction of body fat percent accounted for an additional 7% of the variance in sBRS.

The baroreflex effectiveness was similar across body fat percentiles (P = 0.09), (Table 3).

Autonomic function between late preterm and full-term newborns

Systolic blood pressure and dP/dt_{max} variability

We found no differences in overall blood pressure variability, individual frequency components of blood pressure variability, or in any of the normalized units of low-

Table 2. Maternal and newborn characteristics of full-term and preterm newborns.

	Full term (n = 40)	Preterm (n = 8)	P value
Maternal characteristics			
Age (years)	33 ± 4.2	33 ± 5	0.66
Ethnicity, n (%)			
Asian	8 (21)	3 (38)	
Caucasian	23 (59)	4 (50)	
Middle Eastern	1 (3)	0 (0)	0.82
South Asian	5 (13)	1 (13)	
Other	2 (5)	0 (0)	
Maternal prepregnancy BMI (kg/m^2)	22.9 ± 3.4	24.4 ± 9.2	0.67
Gestational diabetes mellitus, n (%)	6 (15)	2 (25)	0.49
Preeclampsia, n (%)	0 (0)	0 (0)	–
NICU admissions, n (%)	3 (8)	0 (0)	0.42
Glucocorticoid exposure, n (%)	1 (3)	1 (13)	0.20
Maternal smoking, n (%)	0 (0)	0 (0)	–
Hypertension in pregnancy, n (%)	0 (0)	0 (0)	–
Mode of delivery, n (%)			
Normal delivery	23 (58)	6 (75)	
Instrumental	9 (22)	1 (13)	0.65
Cesarean	8 (20)	1 (13)	
Labor			
Spontaneous	22 (55)	6 (75)	
Induced	12 (30)	1 (13)	0.54
No Labor	6 (15)	1 (13)	
Newborn characteristics			
Gestational age (weeks)	39 ± 1.1	36 ± 0.5	<0.0001
Sex (girls/boys)	23/17	3/5	0.30
Birth weight (g)	3387 ± 565	2737 ± 406	0.003
Length (cm)	50 ± 3	47 ± 2	0.012
Head circumference (cm)	35 ± 2	33 ± 2	0.04
Body fat (%)	11 ± 4	7.6 ± 2	0.05
Body fatness (g)	378 ± 183	204 ± 93	<0.01
Fat-free mass (%)	89 ± 4	92 ± 2	0.05
Fat-free mass (g)	3033 ± 423	2515 ± 324	<0.01

Data are presented as mean ± SD for continuous variables using independent t-tests and n (%) for categorical variables, using chi-square tests between full-term and late preterm groups. NICU, neonatal intensive care unit.

and high-frequency components between newborns born late preterm and those born full-term, (Table 4).

We found no differences in overall dP/dt_{max}, dP/dt_{max} variability or individual frequency components of dP/dt_{max} variability between newborns born late preterm and those born full-term. Similarly, the normalized units of low- and high-frequency components were not different between groups, (Table 4).

Baroreflex function

Spontaneous baroreflex sensitivity and baroreflex effectiveness index appeared to be reduced in late preterm newborns compared to full-term newborns; however, this did not reach statistical significance, (Table 4).

Discussion

There is strong epidemiological evidence demonstrating an inverse association between low birth weight and risk of cardiovascular disease in later life (Barker et al. 2005; Huxley et al. 2007). Recently, studies have also identified that being born large for gestational age may also impact cardio-metabolic health (Eriksson et al. 2001; Koklu et al. 2007; Skilton et al. 2014). Being born preterm is associated with hypertension although studies have predominantly focused on severe prematurity, and it is unclear whether those born late preterm show similar cardiovascular maladaptation. One of the major identified modifiers of cardiovascular risk is altered autonomic function (Julius 1991; Parati and Esler 2012). We hypothesized that newborns with high or low body fat, or those born late preterm, may display changes in autonomic function that predispose them to cardiovascular disease, compared to term newborns with normal body fat. However, we found no evidence for altered autonomic modulation of vasomotor function and cardiac contractility at the extremes of the fetal growth spectrum or in newborns born late preterm. The exception was a reduced baroreflex sensitivity in newborns with high body fat, compared to those with normal body fat. This could be a factor that predisposes this group to development of hypertension in later life (Bristow et al. 1969).

Baroreflex function and SBP variability in the newborn

In our study, newborns with HBF showed reduced sBRS compared to those with normal body fat. Studies in obese children and adults show a consistent reduction in baroreflex sensitivity (Skrapari et al. 2007; Lazarova et al. 2009; Calcaterra et al. 2013; Javorka et al. 2013), however, the time of onset of this change is unclear. Our results extend these findings by showing that reduced baroreflex sensitivity is also apparent in newborns with high body fat at just a few days postpartum. Decreased baroreflex sensitivity is a negative prognostic factor for cardiovascular morbidity and sudden cardiac death (La Rovere et al. 1998; Honzíková et al. 2006; Lazarova et al. 2009). Reduced sensitivity may be due to autonomic nervous system dysfunction (Spraul et al. 1994; Chapleau et al. 1995; Miller et al. 1999; Grassi et al. 2004) and or through changes in the mechanical

Table 3. Autonomic indices across body fat percentiles.

	LBF ≤10th percentile (n = 12)	Control >25th to ≤75th percentile (n = 23)	HBF >90th percentile |(n = 7)	P value
Systolic blood pressure variability				
TP ($mmHg^2$)	0.89 (2.98)	0.70 (1.50)	0.82 (1.29)	0.78
LF ($mmHg^2$)	0.49 (1.60)	0.44 (0.95)	0.51 (0.77)	0.99
LF, NU	60.4 ± 16.5	68.5 ± 15.8	74.8 ± 12.6	0.12
HF ($mmHg^2$)	0.25 (1.41)	0.21 (0.34)	0.12 (0.22)	0.58
HF, NU	39.6 ± 16.5	31.5 ± 14.8	25.2 ± 12.6	0.12
dP/dt_{max} variability				
dP/dt_{max} (mmHg/sec)	144.9 (91.94)	105.1 (69.6)	115.9 (45.2)	0.47
TP ($mmHg^2/sec^2$)	9.6 (27.92)	5.8 (19.7)	6.2 (11.0)	0.71
LF ($mmHg^2/sec^2$)	3.2 (6.3)	2.6 (4.5)	3.1 (3.0)	0.91
LF, NU	36.9 ± 15.8	38.3 ± 18.0	39.0 ± 16.3	0.96
HF ($mmHg^2/sec^2$)	5.3 (13.6)	3.1 (10.8)	3.1 (8.8)	0.78
HF, NU	63.1 ± 15.8	61.7 ± 18.0	61.0 ± 16.3	0.96
Baroreflex function				
sBRS (msec/mmHg)	18.4 ± 6.0	20.1 ± 9.4	11.0 ± 6.0	0.04
BEI	0.09 ± 0.05	0.15 ± 0.08	0.13 ± 0.08	0.09

Data presented as mean ± SD for normally distributed data and median (interquartile range) for log-transformed data using one-way analysis of variance (ANOVA). LBP; low body fat percentile, HBF; high body fat percentile, TP, total power; LF, low frequency; HF, high frequency; NU, normalized units; sBRS, spontaneous baroreflex sensitivity; BEI, baroreflex effectiveness index.

properties of the arterial wall (Tanaka et al. 2001; Honzíková et al. 2006). Increased carotid intima-media thickness and stiffness have been found in obese children (Woo et al. 2002; Iannuzzi et al. 2004; Skilton et al. 2014; Park et al. 2015), but there are currently no studies that have investigated the association of arterial stiffness with increased adiposity in the newborn. Reduced sBRS in this group may also be due to increased sympathetic activity as a result of increased plasma insulin or circulating leptin (Lazarova et al. 2009). Leptin has also been known to impair the cardiac baroreflex centrally at the level of the nucleus tractus solitarii (Arnold et al. 2009). We hypothesize that an imbalance in the autonomic nervous system, with an impaired parasympathetic activity alone or with sympathetic over activity may play an important role in the reduced sBRS observed in our newborns with high body fat. Interestingly, across our entire body fat spectrum, we found a nonlinear association between newborn body fat and sBRS, which was independent of birth weight. This may indicate that newborn body fatness may be a better predictor of spontaneous baroreflex sensitivity in the newborn than birth weight.

We found no differences in BPV between body fat groups, (Table 3). The magnitude of BPV in the low-frequency band is regarded as an index of sympathetic modulation of the systemic vasculature and therefore total peripheral resistance (Pagani et al. 1986; Yiallourou et al. 2012a). The role of the high-frequency component of BPV in autonomic regulation is less clear, however, a recent report indicated that it may be linked to respiratory modulation of sympathetic vasomotor tone (Menuet et al. 2017). Although we failed to identify any clear differences in BPV between groups, it is possible that differences may arise in later childhood. Currently there are no studies that have investigated BPV in the neonate or children with high adiposity. It therefore remains to be determined at what age autonomic dysfunction as observed through analysis of BPV at different levels of body fat may manifest.

Low birth weight is strongly, inversely associated with later cardiovascular disease (Barker et al. 2005), but does not discriminate between low birth weight due to fetal growth restriction or prematurity. In our study, newborns born full-term with LBF showed no differences in baroreflex function or BPV. Studies in low birth weight children and adolescents born at term have shown increased BPV, measured in the time domain as the standard deviation from discontinuous noninvasive BP monitoring or standard deviation, coefficient of variation and deviation (Lurbe et al. 2001; Chen et al. 2012). These methodologies do not provide information on autonomic function as does frequency analysis of beat-to-beat BP waveforms and therefore cannot conclude whether the subjects of these studies had altered autonomic regulation. Because low birth weight is a strong predictor of later cardiovascular disease it is important to review whether changes seen in early life are due to prematurity or growth restriction alone, and at what age changes in autonomic

Table 4. Frequency analysis of systolic blood pressure, dP/dt_{max} variability, and baroreflex function in newborns born full term or late preterm.

	Late preterm (34–36 weeks) $n = 8$	Full term 37–42 weeks) $n = 40$	*P* value
Systolic blood pressure variability			
TP ($mmHg^2$)	0.70 (1.99)	0.96 (1.67)	0.24
LF ($mmHg^2$)	0.40 (0.63)	0.70 (1.17)	0.22
LF, NU	65.4 ± 19.0)	69.6 ± 15.0	0.50
HF ($mmHg^2$)	0.21 (1.03)	0.23 (0.35)	0.46
HF, NU	34.6 ± 19.0	30.4 ± 15.0	0.50
dP/dt_{max} variability			
dP/dt_{max} (mmHg/sec)	115.4 (55.2)	120.6 (91.5)	0.94
TP ($mmHg^2/sec^2$)	6.23 (14.51)	5.55 (12.24)	0.80
LF ($mmHg^2/sec^2$)	2.27 (5.85)	2.87 (4.1)	0.75
LF, NU	44.7 ± 17.9	45.0 ± 15.7	0.99
HF ($mmHg^2/sec^2$)	2.7 (7.06)	3.00 (7.42)	0.78
HF, NU	55.3 ± 17.9	55.0 ± 15.7	0.99
Baroreflex function			
sBRS (msec/mmHg)	13.2 ± 7.1	18.0 ± 9.4	0.20
BEI	0.10 ± 0.05	0.2 ± 0.07	0.34

Data presented as mean ± SD for normally distributed data and median (interquartile range) for log-transformed data, independent *t*-test between preterm versus full-term newborns. TP, total power; LF, low frequency; HF, high frequency; NU, normalized units; sBRS, spontaneous baroreflex sensitivity; BEI, baroreflex effectiveness index.

function detrimental to cardiovascular risk may manifest. Future studies measuring BP waveforms in children and adolescents born small for gestational age and late preterm will help to answer this question.

Baroreflex function and SBP variability in late preterm newborns

In our study, although preterm newborns showed a lower percentage body fat and low birth weight compared to their term born counterparts, no differences were observed in baroreflex or vasomotor function between these groups. Studies in newborns born preterm (28–32 gestational age) found reduced baroreflex sensitivity at 2, 3, and 6 months age (Witcombe et al. 2012). Studies by the same authors in children born preterm combined with fetal growth restriction did not affect BPV or baroreflex function; however, children born preterm alone showed increased high-frequency BPV and no differences in baroreflex function (Cohen et al. 2017). It is unclear whether prematurity or growth restriction accounts for these changes seen in these studies. In our study, we did not look to separate fetal growth restriction within those born late preterm.

Arterial dP/dt_{max} variability as an index of autonomic regulation of cardiac contractility

In our study, we found no differences in dP/dt_{max} variability across body fat percentiles or between full-term and late preterm newborns. The dP/dt_{max} of left ventricular pressure is a well-validated measure of contractility (Little 1985). Despite the dP/dt_{max} of the arterial pressure waveform being affected by a number of factors such as preload and arterial compliance (Adler et al. 1996), studies have shown good correlation between ventricular and arterial dP/dt_{max}, and arterial dP/dt_{max} may offer a valuable methodology for noninvasive determination of myocardial contractility (De Hert et al. 2006; Masutani et al. 2009; Morimont et al. 2012).

A major influence on ventricular contractility is the autonomic nervous system (Charkoudian and Rabbitts 2009). This study has introduced a possible new measure, dP/dt_{max} variability as an index of autonomic control of myocardial contractility. We suggest that sympathetic modulation of the ventricular myocardium may be reflected in the low-frequency component of dP/dt_{max} variability in a similar manner to how sympathetic modulation of heart rate and vascular resistance are reflected in the low-frequency components of heart rate variability and BPV (Pagani et al. 1997; Yiallourou et al. 2012a). These factors are not independent, and both heart rate and blood pressure influence dP/dt_{max} because of their influence on preload and afterload. It is unlikely that the force-frequency relationship, where myocardial contractility is observed to increase at higher heart rate, influences dP/dt_{max} variability because these effects are observed when heart rate increases over a sustained time frame (Janssen 2010), rather than with cyclical variations that are the hallmark of the dP/dt_{max} analysis. Moreover, the force-frequency relationship appears to apply more to small mammals than human, at least under normal physiological conditions (Torres and Janssen 2011). Further investigation and validation of dP/dt_{max} variability independent of heart rate variability using specific inotropic and chronotropic agents is important and the subject of future studies. This added index of autonomic regulation of the myocardium may be important in evaluating pediatric and adult patients with autonomic dysfunction.

The use of radial (used in the newborn in our study) or finger arterial pressure derivative and its variability may provide valuable data to monitor cardiac performance noninvasively during daily activities. A limitation of this technique in the newborn is that it is unclear how accurate the radial arterial pressure derivative of dP/dt_{max} is in comparison to gold standard measures of cardiac

contractility. However, as with the use of the Finapres in the newborn for determining BPV, beat-to-beat changes in dP/dt_{max} may be accurate even if the absolute measurement is not (Polson et al. 2006). It is the beat-to-beat change, rather than the absolute value that is important in the determination of dP/dt_{max} variability.

Limitations

Obtaining blood pressure waveform in the newborn noninvasively using photoplethysmography is a technique that offers potentially important advances in both research and clinical settings (Andriessen et al. 2004; Polson et al. 2006; Yiallourou et al. 2012a, 2013; Cohen et al. 2017). However, the methodology is technically challenging, and often investigators are unable to obtain adequate blood pressure signals in subjects. Moreover, the accuracy of the measurements has been questioned (Drouin et al. 1997a, b; Andriessen et al. 2004; Polson et al. 2006). We have found previously that with appropriate cuff placement, there is good agreement between photoplethysmography and an arterial cannula in mean diastolic and systolic blood pressure measurements made over several minutes, however, on a beat-to-beat basis absolute measures of blood pressure were not well validated (Polson et al. 2006). Importantly, however, we found that measurement of the beat-to-beat changes in systolic blood pressure, and therefore calculations of systolic BPV and spontaneous BRS, were accurate (Polson et al. 2006).

This study is part of a wider study assessing early cardiovascular risk which included measures of heart rate variability obtain from the ECG, as another marker of autonomic control. However, due to the technical difficulties in measuring blood pressure waveform in the newborn, we were only able to obtain continuous blood pressure waveform data in a subset of participants. Thus, the sample size in the present report is reduced compared to heart rate variability measures. Given the large difference in the number of participants with available data, measures of heart rate variability were not included in this study and will be published elsewhere in conjunction with other markers of cardiovascular risk. The sample size in this study is, however, similar to, or higher than, other studies that have assessed autonomic function in the infant (Andriessen et al. 2004, 2005; Patural et al. 2004; Polson et al. 2006).

Conclusions

There is strong evidence demonstrating that babies born at the extremities of the birth weight spectrum are at increased risk of developing cardiovascular disease in later life, although the mechanisms remain unclear. Moreover, the age at which predisposition for cardiovascular disease can be demonstrated is not known. In this study, we sought to ascertain whether babies born with high or low body fat, or those born late preterm, showed changes in autonomic function, compared to controls. We found little evidence for altered autonomic function of vasomotor function and cardiac contractility in these groups, with the exception of newborn with high body fat who showed reduced baroreflex sensitivity. Reduced baroreflex sensitivity observed in newborns with high body fat may be due to impaired parasympathetic activity or sympathetic over activity. Furthermore, across the entire body fat spectrum ($n = 62$), there was a nonlinear association between newborn body fat and baroreflex sensitivity which was independent of birth weight ($P = 0.04$). This study has introduced dP/dt_{max} variability as a possible new measure of autonomic control of myocardial contractility. This novel index, proposed as a measure of autonomic regulation of the myocardium, may be of value in evaluating pediatric and adult patients with autonomic dysfunction. Further investigation and validation of dP/dt_{max} variability using specific inotropic and chronotropic agents is important and the subject of future studies.

Conflict of Interest

None.

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CHAPTER FIVE

BODY FATNESS AND CARDIOVASCULAR HEALTH IN NEWBORN INFANTS

Original Article

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ABSTRACT

Importance: Birth weight is associated with cardiovascular disease, with those at both ends of the spectrum at increased risk. Markers of cardiovascular risk are altered in both low and high birth weight infants. However, birth weight is a relatively crude surrogate of fetal growth, and it has been proposed that body composition of newborns, particularly body fatness, may more accurately identify "high risk" infants.

Objective: Determine whether cardiac autonomic control, and cardiac structure and function differ in newborns with high or low body fatness compared to those with average body fatness.

Design, Setting, and Participants: A cross-sectional study was conducted between 2015-2017 at Royal Prince Alfred Hospital, Sydney, Australia. 189 healthy singleton term born neonates were recruited from the maternity wards. Recruitment was stratified by predefined groups on the basis of body fat percentiles (sex and gestation-specific). Our prespecified analyses were comparisons of High body fatness (>90th percentile) and Low body fatness (≤10th) with Control (>25 to ≤75th).

Main outcomes and measures: Outcome measures included were heart rate variability as a measure of cardiac autonomic control, and cardiac structure and function assessed by echocardiography. Body fat was assessed by air displacement plethysmography.

Results: Infants with low body fat had lower heart rate variability (log, total power, -0.5 [95% CI -0.8, -0.1, P = .008), and thicker ventricular walls (posterior wall thickness normalised to body surface area, 3.1 mm [95% CI 1.6, 4.6], P <0.001) compared to controls. Infants with high body fat showed reduced heart rate variability (log, total power, -0.8 [95% CI -1.1, -0.5], P < 0.001) and stroke volume (-0.3 ml/kg [95% CI -0.6, -0.0], P = .03). The non-linear association of body fatness with heart rate variability was independent of birth weight.

Conclusions and relevance: Infants born with high or low body fat have altered markers of cardiovascular health and may represent groups at higher risk of future cardiovascular disease.

INTRODUCTION

Cardiovascular disease remains the leading cause of death globally, accounting for 15 million deaths in 2015 [1]. There is an extensive and consistent body of epidemiologic evidence supporting an inverse association of birth weight with incidence of adult cardiovascular disease, such that for every 1kg higher birth weight, the risk of coronary artery disease is 10-20% lower [2]. However, this association is likely not linear, with people born with a high birth weight, also exhibiting evidence of altered cardio-metabolic health in childhood and adulthood, [3-5] and there is indirect evidence supporting a higher risk of subsequent cardiovascular disease events [2].

Although cardiovascular disease presents clinically in adult life, evidence of structural and functional changes to the vasculature consistent with the earliest etiologic features of atherosclerosis are present in in infants with early life risk factors, [6], such as low birth weight [7] [8].

These early physiological markers of cardiovascular risk are increasingly well described, and include cardiac structural and functional modification, and alterations in cardiac autonomic control. Studies have shown altered cardiac morphology and subclinical impairments of systolic and diastolic cardiac function in small for gestational age (SGA) infants,[9, 10] which persist into childhood [11] and late adolescence [12]. By mid-adulthood, the magnitude of these effects would appear to be of limited clinical relevance [13]. But even in the context of this presumed reversal by mid-adulthood, such temporary structural and functional cardiac impairments during the first two decades of life may theoretically predispose an individual to later cardiovascular disease via prolonged exposure to hemodynamic alterations, accelerating the onset and progression of arteriosclerosis and causing subclinical end-organ damage.

Such alterations in cardiac function may etiologically relate to changes in cardiac autonomic control. The physiological variation in the heart rate is modified by the sympathetic and parasympathetic branches of the autonomic nervous system which act together to regulate blood pressure and cardiovascular function starting in fetal life. Autonomic activity is an important

mechanistic determinant of other health and disease outcomes [14]. Heart rate variability (HRV), a surrogate of cardiac autonomic control, can be assessed non-invasively, and is associated with risk of subsequent cardiovascular events [15]. There is evidence that cardiac autonomic control is altered in SGA infants from the prenatal period through to childhood and adult life [16].

The vast majority of this body of evidence linking fetal growth with adult cardio-metabolic disease, and markers of pre-clinical disease in childhood, uses birth weight as the exposure of interest, as it is easily obtained and considered a useful, albeit rather crude, surrogate marker of fetal growth and nutrition. However, birth weight and birth weight percentiles are unable to differentiate between the constitutionally small but well-nourished infant who has met their genetic growth potential, from malnourished infants of the same weight with evidence of fetal growth restriction. Similarly, those who are large for gestational age may be constitutionally large or have excessive weight gain. It has been proposed that the groups of infants with restricted or excessive growth in response to their intrauterine environment are those with increased rates of adult cardiovascular disease,[17] although the use of clinically applied measures of such have failed to show consistent associations with cardio-metabolic changes consistent with high risk [18, 19]. One possible means by which to quantify newborn body composition is through the amount of lean and fat body mass. Lean body mass is relatively stable and largely reflects genetic and familial determinants, such as parental size [20]. Newborn fat mass is more variable and appears to reflect the intrauterine environment, including nutritional status of the mother [20-22].

Accordingly, we sought to determine whether autonomic control and cardiac structure and function, differ in newborns with either high or low body fatness, when compared to those with “normal” body fatness. Furthermore, we aimed to identify whether BF% is a better predictor than birth weight of any such alterations in cardiac autonomic control, structure and function.

METHODS

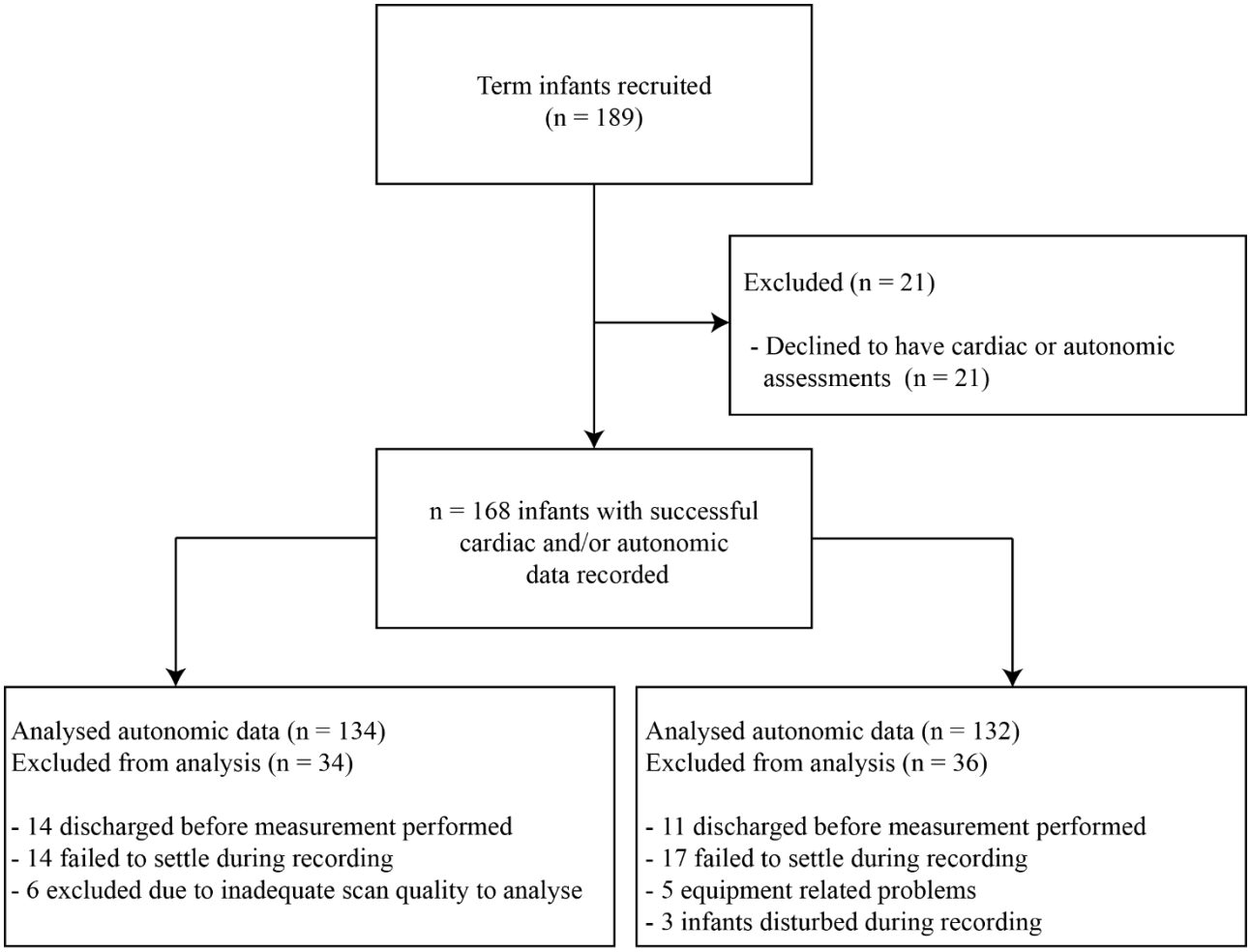
This study was approved prospectively by the Sydney Local Health District ethics committee and the University of Sydney Human Ethics committee. Participation was voluntary, and informed written consent was obtained for all participants.

The study comprised two parts. First, acquiring ECG data in quietly resting neonates for analysis of HRV, as an index of autonomic regulation of the heart. Second, ultrasound examination of the structure and function of the heart.

Subject Recruitment

Recruitment consisted of a total of 189 newborns from the postnatal wards and the neonatal unit at Royal Prince Alfred Hospital, Sydney. This is a major obstetric tertiary referral centre with approximately 5500 deliveries per year, and covers an inner-city, multicultural population. Newborns were recruited consecutively according to consent within the body fat percentile categories and according to gender balance within each subgroup. Eligible subjects were well singleton newborns between 37 and 42 completed weeks gestation who had undergone routine body composition measurements. Exclusions were major congenital abnormalities and ongoing need for respiratory support. Gestational age was calculated from first trimester ultrasounds.
Recruitment and Study flow is shown in figure 1.

Figure 1: study flow



Body composition and anthropometry

Body composition was measured in infants in the first 48 hours postpartum using air-displacement plethysmography (PEA POD®, COSMED USA, Inc), as part of their routine clinical management. Air displacement plethysmography accurately measures body volume by the application of Boyle's law to the displacement of air by the infant in a sealed chamber, with proprietary algorithms used to calculate fat mass and fat-free mass [23-25]. Infants were categorised according to published body fat percentiles [26] Birth weight, length and head circumference were measured concurrently by two trained midwives. Weight was measured with the integrated PEA POD® scales to the nearest gram, and head circumference to 0.1cm. Length was measured to the nearest 0.1cm with an Easy-Glide Bearing Infantometer (Perspective Enterprises, USA) length board.

Maternal and infant demographics

Demographic data on mothers and infants were collected from mothers using a standardised questionnaire and from mother and infant medical records. We collected current and previous health status, pregnancy and delivery details, including previous medical conditions, gestational diabetes, gestational hypertension, smoking status, physical activity and dietary profile.

Heart rate variability

ECG was recorded continuously for 15 minutes using standard neonatal 3-lead configuration whilst the infants were sleeping in a supine position. The ECG analog output was digitised at 500 Hz, and acquired using commercial hardware (Powerlab, ADInstruments, Sydney, Australia). Infants behaviour was observed closely; any periods of activity where the infant woke up were noted and these periods removed from subsequent analysis. Analysis of heart rate (HR) and HRV were performed using LabChart (HRV 1 module, version 7, ADInstruments) on up to three (average 2.75) R-R interval epochs of exactly 4 minutes. Peak detection on ECG was used to create RR sequences. Time domain measures of HRV included total power the standard deviation of the normal-to-normal (NN) RR intervals as a measure of overall variability, and two short term measures: standard deviation of change in successive NN intervals (SDΔNN), and the root mean square of successive differences in NN interval (RMSSD) [15]. Frequency domain analysis was done by performing a fast Fourier transformation (256 point, Hanning window) with 50% overlap. The spectral bands for HRV were investigated in the range of 0-1.1 Hz based on previous studies [27, 28]. Low Frequency (LF) at 0.04 to 0.15 Hz, and high frequency (HF) at 0.15 to 1.1 Hz. The HF band was based on respiratory rates in infants at 0.5 to 1 Hz [29, 30]. VLF was not determined due to the short sampling times.

Cardiac Ultrasound

2D cardiac ultrasound was performed with a Philips EPIQ 5 and S12-4 Phased Sector Array transducer using a standardised protocol, from day 2 after birth to allow for postpartum cardiovascular transition. Images were stored and analysis performed offline with Xcelera software V (Philips Medical Systems, Netherlands).

Cardiac dimensions: Left and right ventricle base to apex length and diameter were measured in apical 4 chamber view at end-diastole, and sphericity index calculated as length/diameter. Left ventricular wall thickness was measured at end-diastole just distal to the mitral leaflets in M-mode from a parasternal long axis view.

Systolic function: Fractional shortening was measured in M-mode from a parasternal long axis view and calculated as end-diastolic diameter – (end-systolic diameter/end-diastolic diameter). Ascending aortic velocity time integral was measured from an apical 5 chamber view with pulsed-wave Doppler. Internal aortic diameter was measured at parasternal long axis view where the aortic valve leaflets were clearly visualized. Stroke volume was calculated as aortic area x heart rate and left ventricular output as aortic area x velocity time integral x heart rate. Mitral and tricuspid annular plane systolic excursion were measured using M-mode through respective lateral valve leaflets from apical 4 chamber view.[31] Peak systolic myocardial velocity was measured with tissue Doppler imaging at the lateral and septal mitral and lateral tricuspid valves utilising pulsed-wave Doppler from the apical 4 chamber view.[32]

Diastolic function: Ventricular inflow was measured using pulsed-wave Doppler of the atrioventricular valves from an apical 4 chamber view. Peak velocities of the E and A waves were measured and used to calculate the E: A ratio. E deceleration time was calculated as time from peak velocity of the E wave to the return to baseline. Isovolumetric relaxation time (IVRT) was

measured from an apical view, with the sample volume placed to simultaneously record both mitral and aortic flow. IVRT was calculated as the end of flow through the aortic valve to the beginning of flow through the mitral valve. Peak diastolic myocardial velocities (E′ and A′) were measured with tissue Doppler imaging at the lateral and septal mitral and lateral tricuspid valves utilising pulsed-wave Doppler from the apical 4 chamber view. Lateral and septal E: E′ were calculated.

To determine intra-observer variability, blinded analyses were performed on two separate occasions at least one month apart for 20 randomly selected participants. Two anatomical and two physiological parameters were analysed utilising the coefficient of variation and the intra-observer intra-class correlation coefficient. Intra-class correlations ranged from 0.75 to 0.98 (Supplementary Table 1).

Statistical analysis

Continuous data are expressed as mean (SD) and categorical data as n (%). Data were tested for normality by plotting histograms, and log transformations applied as required. Log transformed data are presented as the geometric mean (interquartile range). Multivariable linear regression were used to compare HBF (>90th percentile) and LBF (≤10th percentile) infants with a control group of infants with average body fatness (>25th to ≤75th percentile). Chi–Square or Fisher's Exact tests were utilised for categorical data. Nonlinear associations were determined by use of quadratic terms in multivariable regression models that included participants across the entire spectrum of body fatness. Statistical significance was inferred at 2P<0.05. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, NY).

RESULTS

Participant characteristics

Maternal and infant characteristics are shown in (Table 1). Mothers of infants with LBF were more likely to identify as South Asian and less likely to identify as Caucasian, and maternal BMI was higher in mothers who gave birth to infants with HBF compared to infants in the control group. Infants with HBF were more likely to be delivered by elective caesarean section and presented with no labour, a greater proportion of control infants had a normal delivery with spontaneous onset of labour. There were no mothers on glucocorticoid intervention and prevalence of maternal smoking did not differ between test groups.

As expected based on selection criteria, infants with LBF were lighter, shorter and had a reduced head circumference compared to controls, and infants with HBF were heavier, taller and had a larger head circumference. Maternal and infant characteristics for those infants between the >10th and ≤25th body fat percentiles, and between the >75th and ≤90th body fat percentiles are provided in (Supplementary Table 2).

Heart rate variability

Heart rate was similar across body fat percentiles (Table 2). Measures of overall HRV both in the time (SDNN) and frequency domain (total power) were lower in LBF and HBF than control infants (Table 2). These associations remained statistically significant after adjustment for gestational age and sex (eg. for log, total power: LBF, -0.5 [95% CI -0.8, -0.1], P = .008; HBF, -0.8 ms^2 [95% CI -1.1, -0.5], P < 0.001; both compared to control).

Furthermore, SDΔNN and RMSSD, both short term time domain measures, and the HF and LF power measures were all lower in HBF infants than controls (Table 2).

Across the entire body fat percent spectrum, there was a non-linear association between infant body fat percent and overall HRV (total power, figure 2; $P = .001$, adjusted for gestational age and sex), which was independent of birth weight ($P = .01$ after adjustment). Body fat percent accounted for 8.6 % of the variance in overall HRV. In comparison, birth weight accounted for 8.2% of the variance in overall HRV. In a model which included birth weight, the introduction of body fat percent accounted for an additional 4.7% of the variance in overall HRV. Absolute heart rate variability indices across body fat percentiles shown in supplementary table 3.

Cardiac structure and function

Infants with LBF had significantly increased septal and posterior wall thicknesses and left ventricular chamber dilatation compared to control infants (Table 2). Physiological parameters were similar between LBF and control infants (Supplementary Table 4). Infants with HBF had increased ventricular base to apex length (Table 2) and lower left ventricular stroke volume compared to control infants (Supplementary Table 4), with no other differences observed in cardiac structure or measures of systolic or diastolic function.

There were no statistically significant associations, linear or non-linear, between cardiac measures and body fat percent across the entire spectrum of body fat measures (results not shown). Six infants had a small ventricular septal defect or patent ductus arteriosus, which were asymptomatic with minimal shunt. There was no difference in incidence between groups, and sensitivity analyses indicated that results did not differ when these infants were excluded. Absolute cardiac structure and function indices across body fat percentiles are shown in supplementary table 5.

Table 1: Maternal and infant characteristics

	Control (>25th to ≤75th BF%) (n = 56)	LBF (≤10th BF%) (n = 33)	P value (LBF vs control)	HBF (>90th BF%) (n =29)	P value (HBF vs control)
Maternal Characteristics					
Age, years	33 ± 4.2	32 ± 3.9	0.31	34.8 ± 4.6	0.17
Ethnicity, n (%)					
Asian	11 (20)	5 (16)		4 (14)	
Caucasian	39 (70)	14 (45)		22 (76)	
Middle Eastern	1 (2)	2 (7)	0.04	0 (0)	0.81
South Asian	3 (5)	7 (23)		1 (3)	
Other	2 (4)	3 (10)		2 (7)	
Maternal pre-pregnancy BMI, Kg/m^2	22.6 ± 3.7	22.1 ± 2.7	0.47	24.6 ± 3.6	0.03
Gestational Diabetes Mellitus, n (%)	11 (20)	5 (15)	0.59	3 (10)	0.27
Hypertension in pregnancy, n (%)	1 (3)	2 (4)	0.89	0 (0)	0.30
Mode of delivery, n (%)					
Normal delivery	35 (63)	20 (61)		13 (45)	
Instrumental	12 (21)	7 (21)	0.97	3 (10)	0.01
Caesarean	9 (16)	6 (18)		13 (45)	
Labour					
Spontaneous	34 (61)	17 (52)		10 (35)	
Induced	18 (32)	13 (39)	0.70	6 (21)	<0.001
No Labour	4 (7)	3 (9)		13 (45)	
Infant Characteristics					
Postnatal age, days	2 ± 1	2 ± 2	0.22	2 ± 1	0.90
Gender (girls/boys)	32/24	18/15	0.81	14/15	0.44
Birth weight, g	3401 ± 415	2916 ± 360	<0.0001	3997 ± 437	<0.0001
Length, cm	49.8 ± 1.9	47.6 ± 1.8	<0.0001	51.6 ± 1.9	<0.0001
Head circumference, cm	34.6 ± 1.3	33.8 ± 1.2	<0.001	36.0 ± 1.3	<0.0001
Body fat, %	10.6 ± 1.8	3.9 ± 1.8	<0.0001	17.9 ± 2.5	<0.0001

Data are presented as mean (SD) for continuous variables using Independent t-test and n (%) for dichotomous variables using chi-square tests between groups. LBF, low body fat; HBF, high body fat. Control = 56; LBF = 33; HBF = 29 except BMI, n = 32 (LBF), n = 54 (control); maternal age n = 25 (HBF).

Table 2. Effect of infant body fat percent on aortic intima-media thickness, heart rate variability and cardiac structure

	Control (>25th to ≤75th BF%)	LBF (≤10th BF%)		HBF (>90th BF%)	
	β (95% CI)	*β* (95% CI)	*P* value	*β* (95% CI)	*P* value
HRV (frequency domain)	**N = 39**	**N = 25**		**N = 26**	
Ln Total Power	0 (referent)	-0.5 (-0.8, -0.1)	.01	-0.8 (-1.1, -0.5)	< 0.001
Ln LF	0 (referent)	-0.3 (-0.7,0.1)	.18	-0.8 (-1.1, -0.4)	< 0.001
Ln HF	0 (referent)	-0.2 (-0.7,0.3)	.51	-1.0 (-1.4, -0.4)	< 0.001
Ln LF: HF	0 (referent)	-0.2 (-0.6,0.2)	.38	0.3 (-0.1,0.8)	.15
HRV (time domain)	**N = 39**	**N = 25**		**N = 26**	
HR, bpm	0 (referent)	-0.5 (-9,8)	.92	4 (-4,12)	.32
Mean NN, ms	0 (referent)	11 (-22,44)	.48	-18 (-50,13)	.25
SDNN, ms	0 (referent)	-7 (-13, -1)	.02	-11 (-17, -5)	< 0.001
Ln SDΔNN	0 (referent)	-0.1 (-0.4,0.2)	.52	-0.5 (-0.7, -0.2)	.02
Ln RMSSD	0 (referent)	-0.1 (-0.4,0.2)	.39	-0.4 (-0.7, -0.1)	.004
Cardiac structure	**N = 46**	**N = 22**		**N = 23**	
LV Base to apex length, mm	0 (referent)	-1.34 (-3.1,0.3)	.11	2.09 (0.3, 3.8)	.02
LV Diameter, mm	0 (referent)	-0.88 (-2,0.3)	.13	0.09 (-1.1,1.3)	.89
LV Sphericity index	0 (referent)	0.05(-0.1,0.2)	.46	0.18 (-0.02,0.3)	.09
Septal wall thickness/BSA	0 (referent)	2.3 (0.5,4.1)	.01	-0.01 (-1.8,1.7)	.99
Posterior wall thickness/BSA	0 (referent)	3.1 (1.6,4.6)	<0.001	-1 (-2.4,0.5)	.18
End-diastolic dimension/BSA	0 (referent)	5.5 (1.4,9.7)	.01	-3 (-7.1,1.0)	.14
Relative wall thickness	0 (referent)	0.03 (-0.01,0.7)	.08	-0.01 (-0.04,0.3)	.71
RV Base to apex length, mm	0 (referent)	-0.8 (-2.6,1.0)	.36	2.0 (0.36,3.7)	.02
RV Diameter, mm	0 (referent)	-0.7 (-1.7,0.35)	.19	0.4 (-0.5,1.4)	.38
RV Sphericity index	0 (referent)	0.01 (-0.1,0.2)	.87	0.09 (-0.1,0.2)	.23

Values are unstandardized β-regression coefficients (95% CI) from multivariable models, adjusted for sex and gestational age. Ln, log transformed data.

LBF: low body fat percent; HBF: high body fat percent; HRV, heart rate variability; LF, low frequency; HF, high frequency; LF: HF, low frequency/high frequency ratio; HR; heart rate, mean NN; mean of N wave to N wave variation normal; SDNN, the mean of the standard deviation of all normal RR intervals; SDΔNN, SD change in NN; RMSSD, square root of the mean squared differences of successive NN intervals; LV, left ventricle; RV, right ventricle; BSA, body surface area.

DISCUSSION

Our findings indicate that infants with LBF have reduced cardiac autonomic control and altered cardiac structure, in particular having thicker ventricular walls and larger chamber size. At the other end of the body fatness spectrum, infants with HBF also showed reduced cardiac autonomic control, although this was accompanied by a different cardiac phenotype, specifically cardiac functional changes, including reduced stroke volume, but not structural changes.
Furthermore, when compared to birth weight, the associations of body fatness with cardiac autonomic control were stronger, suggesting that the use of body fatness assessment may better identify those at risk of cardiovascular diseases than the use of birth weight alone.

More precise identification of individuals at high risk of cardiovascular disease remains a priority in prevention initiatives. Early life factors are increasingly recognized as being associated with future risk of disease, however, they remain crudely described. A number of studies have now described altered autonomic functions to be present in people born with low birth weight, from infancy through adult life [16, 33, 34]. Spassov *et al* found reduced HRV at 2-10 days of age in SGA newborns [35], Galland *et al* found increased sympathetic activity and reduced parasympathetic activity at 1 and 3 months of age [36], whereas Cohen *et al* found no differences in resting HRV [37, 38] despite increased sympathetic response at 0-2 weeks [37]. We extend these previous findings by characterising infants based on their body fatness profile at birth, potentially a more relevant marker of an adverse fetal environment than birth weight. We found that infants with LBF had lower overall HRV (total power and SDNN), and that total power of HRV was independent of birth weight. Accordingly, reduced HRV may indicate a reduced ability to adapt to internal and external stimuli making these individuals mechanistically susceptible to future hemodynamic and cardiac diseases, particularly via autonomic nervous system pathways.

In our study, infants with HBF showed a marked reduction in HRV, seen in all frequency and time domain measures. Studies in obese children and adults show changes to autonomic function consistent with a reduced parasympathetic and increased sympathetic modulation to the heart [39-42]. Previous literature on cardiac parasympathetic response to higher body fatness and obesity in children and adults are remarkably consistent. Children over 6 years of age with obesity show reduced parasympathetic activity, specifically a reduction in HF power is seen in children with obesity during 5 minute recordings [43], 24 hour Holter monitoring [44-46] and during a Valsalva manoeuvre [47]. Other studies in children reported a reduction in Total power, LF and HF power in children with obesity compared to those without, or a reduction in LF and HF power in children with diabetes mellitus and increased body weight [43, 48]. Consistent with the literature in children, our study showed that infants with high body fatness had reduced overall HRV (total power and SDNN), reduced LF, and a clear reduction in parasympathic modulation indicative of a reduced HF power, RMSSD, and SD Δ NN. However, no differences were seen in the LF: HF ratio, a marker of cardiac sympathetic drive. Collectively, the marked reduction in HRV seen in infants with high body fatness may indicate an overall reduction in parasympathetic modulation to the heart. This may be precipitated by both maternal factors and other intrauterine exposures. Our study did not look to elucidate these specific mechanistic pathways that link adiposity and HRV, although they may involve fetal glucose, insulin and adipocytokine levels.

These reductions in HRV in infants with LBF or HBF may potentially be mechanistically involved in the higher risk of stillbirth at the extremes of the fetal growth spectrum, although further work would be required to develop and test this hypothesis.

The most commonly reported structural adaptation that accompanies fetal growth restriction is a globular ventricle, as assessed by sphericity index [9, 49]. Whilst we did not observe a significant difference in sphericity index in the LBF group, we found that infants with LBF have thicker ventricular walls and larger chamber size, which is consistent with the general structural adaptations

previously described. These changes have been demonstrated to be related to the severity of growth restriction as defined by body weight and antenatal Doppler assessment. Interestingly, we found no added discriminatory benefit of using body fatness for identifying infants with these cardiac adaptations, beyond the use of birth weight alone. This hypertrophic phenotype of fetal origin persists into adolescence [11, 50, 51] and adulthood [13], although the cardiac phenotype becomes more subtle with age. Whether the associations of body fatness with this cardiac phenotype will persist, strengthen, or weaken over time remains unknown. Accompanying this structural phenotype are subtle changes in diastolic and systolic function.

A key strength of our study is that we investigated infants at a very early age, on average 3-5 days old. The observation that cardiac autonomic, structural and functional changes seen are already established in the infant provides further insight into the cardiovascular adaptations that may be useful in assessing the effects of exposure to an adverse intrauterine environment. Future follow up of these infants will allow us to determine whether these changes seen in infancy persist into later life, or whether they are transient in infancy.

Our study has a number of limitations. Although our measurements were taken during sleeping, studies have shown that HRV differs by sleep state (active vs quiet sleep), consistent with a greater cardiac sympathetic presence during active sleep. We did not confirm sleep state (active vs quiet sleep) via polysomnography, which may contribute to increased variability in HRV measures.

All participants in our study did not have complete cardiac structural measures and autonomic data therefore it was hard to make associations between changes seen.

Collectively, infants born with low or high body fatness show reduced HRV, an established marker of autonomic activity, although these groups have distinct cardiac structural and functional phenotypes. The immediate or long-term consequences of impaired autonomic function are unknown in the infant, particularly in the context of concurrent cardiac structural and functional

alterations, but in older people are associated with risk of cardiovascular, lipid and endocrine abnormalities, and may reflect a compromised ability of these infants to adapt to ongoing internal or external stimuli.

Supplemental methods and table.

Cardiac dimensions: Left and right ventricle base to apex length and diameter were measured in apical 4 chamber view at end-diastole, and sphericity index calculated as length/diameter. Left ventricular wall thicknesses were measured at end-diastole just distal to the mitral leaflets in M-mode from a parasternal long axis view. Relative wall thickness was posterior wall thickness + septal thickness/left ventricular end diastolic dimension. Wall thicknesses were indexed to body surface area as calculated by the Haycock formula.[52]

***Systolic function*:** Fractional shortening was measured in M-mode from a parasternal long axis view, and calculated as end-diastolic diameter – (end-systolic diameter/end-diastolic diameter). Ascending aortic velocity time integral was measured from an apical 5 chamber view with pulsed-wave Doppler. Internal aortic diameter was measured at parasternal long axis view where the aortic valve leaflets were clearly visualized. Stroke volume was calculated as aortic area x heart rate, and left ventricular output as aortic area x velocity time integral x heart rate. Mitral and tricuspid annular plane systolic excursion were measured using M-mode through respective lateral valve leaflets from apical 4 chamber view. Peak systolic myocardial velocity was measured with tissue Doppler imaging at the lateral and septal mitral and lateral tricuspid valves utilising pulsed-wave Doppler from the apical 4 chamber view.

***Diastolic function*:** Ventricular inflow was measured using pulsed-wave Doppler of the atrioventricular valves from an apical 4 chamber view. Peak velocities of the E and A waves were measured and used to calculate the E:A ratio. Isovolumetric relaxation time was measured from an apical view, with the sample volume placed to simultaneously record both mitral and aortic flow, and calculated as the end of flow through the aortic valve to the beginning of flow through the mitral valve. Peak diastolic myocardial velocities (E′ and A′) were measured with tissue Doppler imaging at the lateral and septal mitral and lateral tricuspid valves utilising pulsed-wave Doppler from the apical 4 chamber view. Lateral and septal E: E′ were calculated.

Supplementary Table 1. Intra-class correlation

	Coefficient of variation	ICC (95% CI)
Left ventricle end-diastolic diameter	3.85	.93 (0.81-0.97)
Left ventricle end-systolic diameter	4.0	.75 (0.47-0.89)
Ascending aorta velocity time integral	3.73	.98 (0.95-0.99)
Left ventricular output	15.1	.82 (0.57-0.92)

Supplementary Table 2: Maternal and infant characteristics for infants in >10th and ≤25th and >75th and ≤90th groups

	>10th and ≤25th (n = 23)	>75th and ≤90th (n = 27)
Maternal Characteristics		
Age (years)	33 ± 5	33 ± 4
Ethnicity, n (%)		
Asian	5 (23)	3 (11)
Caucasian	12 (55)	21 (78)
Hispanic	1 (5)	0 (0)
Middle Eastern	1 (5)	1 (4)
South Asian	3 (14)	2 (7)
Other	0 (0)	0 (0)
Maternal pre-pregnancy BMI, Kg/m^2	24 ± 4	24 ± 4
Gestational Diabetes Mellitus, n (%)	1 (4)	5 (19)
Hypertension in pregnancy, n (%)	1 (4)	0 (0)
Mode of delivery, n (%)		
Normal delivery	14 (61)	17 (63)
Instrumental	5 (22)	2 (7)
Caesarean	4 (17)	8 (30)
Labour		
Spontaneous	14 (63)	16 (59)
Induced	8 (35)	6 (22)
No Labour	1 (4)	5 (19)
Infant Characteristics		
Postnatal age, days	3 ± 1	3 ± 2
Gender, girls/boys	10/13	14/13
Birth weight, g	3083 ± 266	3676 ± 341
Length, cm	50 ± 2	50 ± 2
Head circumference, cm	34 ± 1	35 ± 1
Body fat, %	7 ± 1	15 ± 2

Data are presented as mean (SD) for continuous variables and n (%) for dichotomous variables. Independent t-test and chi-square tests between groups.

Supplementary Table 3. Heart rate variability indices across body fat percentiles

	Control (>25th to ≤75th BF%)	LBF (≤10th BF%)	*P* value (LBF vs control)	HBF (>90th BF%)	*P* value (HBF vs control)
Heart rate variability	**N = 39**	**n = 25**		**n = 26**	
Frequency domain					
*Total Power, ms^2	1556 (1312.6)	960 (1226.3)	.01	702 (552.8)	< 0.001
*LF, ms^2	374 (384.2)	288 (318.1)	.18	176 (152.1)	< 0.001
*HF, ms^2	196 (301.0)	157 (160.9)	.40	79 (92.6)	< 0.001
*LF: HF	2.3 (2.3)	2.0 (2.7)	.49	3.1 (2.1)	.21
Time domain					
HR, bpm	128 (17)	128 (20)	.92	132 (14)	.30
Mean NN, ms	477 (60)	488 (78)	.55	460 (48)	.22
SDNN, ms	44 (13)	36 (12)	.02	33 (10)	< 0.001
*SD∆NN, ms	19 (19.2)	17 (13.3)	.40	12 (8.9)	.002
*RMSSD, ms	19 (19.2)	17 (10.1)	.31	13 (8.9)	.01

Data presented as mean (SD) or *geometric mean (IQR) for log transformed data. Independent t-test between body fat percentile groups. LBF: low body fat percent; HBF: high body fat percent; LF, low frequency; HF, high frequency; LF: HF, low frequency/high frequency ratio; HR; heart rate, mean NN; mean of N wave to N wave variation normal; SDNN, the mean of the standard deviation of all normal RR intervals; SD∆NN, SD change in NN; RMSSD, square root of the mean squared differences of successive NN intervals

Supplementary 4. Indices of cardiac function in infants with low, high and control body fat.

	Control (>25th to ≤75th BF%)	LBF (≤10th BF%)		HBF (>90th BF%)	
	β (95% CI)	β (95% CI)	*P* value	β (95% CI)	*P* value
Systolic function	N = 46	N = 20		N = 19	
Heart rate	0 (referent)	0.8 (-11,12)	.90	7.6 (-4,19)	.21
Fractional shortening, %	0 (referent)	-0.2 (-3.8,3.3)	.70	0.9 (-2.5,4.4)	.60
Left ventricular output, ml/kg/min	0 (referent)	-10.6 (-46,25)	.56	-25 (-60,11)	.17
Stroke volume, ml/kg	0 (referent)	-0.1 (-0.4,0.1)	.33	-0.3 (-0.6,-0.03)	.03
MAPSE, mm	0 (referent)	-0.4 (-1,0.3)	.29	0.02 (-0.1,0.1)	.56
TAPSE, mm	0 (referent)	-0.1 (-0.1,0.1)	.77	0.1 (-0.01,0.2)	.07
Mitral lateral S', cm/s	0 (referent)	0.02 (-0.5,0.5)	.94	0.4 (-0.1,1.0)	.12
Mitral septal S', cm/s	0 (referent)	0.01 (-0.4,0.4)	.98	-0.7 0.9(-0.5,0.4)	.78
Tricuspid S', cm/s	0 (referent)	-0.2 (-0.7,0.2)	.34	-0.3 (-0.9,0.3)	.28
Diastolic function	N = 37	N = 21		N = 15	
Mitral E wave, cm/s	0 (referent)	0.9 (-5.4,7.2)	.78	4.5 (-2.5,11.5)	.20
Mitral A wave, cm/s	0 (referent)	1.9 (-4.2,8.0)	.54	4.2 (-2.6,11.0)	.22
Mitral E:A	0 (referent)	-8.8 (-27,9)	.34	-8.2 (-28,12)	.42
Tricuspid E wave, cm/s	0 (referent)	-2.5 (-8.8,3.8)	.43	6.1 (-0.8,13)	.08
Tricuspid A wave, cm/s	0 (referent)	-0.5 (-5.6,4.6)	.85	5.9 (0.3,11.5)	.04
Tricuspid E:A	0 (referent)	-0.05 (-0.1,0.2)	.19	0.01 (-0.1,0.1)	.72
Left isovolumic relaxation time, ms	0 (referent)	-3.6 (-8.7,1.0)	.12	-2.7 (-8.0,2.7)	.32
Mitral lateral E', cm/s	0 (referent)	-0.7 (-1.5,1.5)	.11	0.01 (-1,1)	.99
Mitral septal E', cm/s	0 (referent)	-0.1 (-1,0.6)	.75	0.6 (-0.2,1.5)	.14
Tricuspid E', cm/s	0 (referent)	-1.1 (-2,-0.07)	.04	-0.5 (-1.6,0.7)	.43
E:E' lateral	0 (referent)	0.8 (-0.7,2.3)	.29	0.9 (-0.8, 2.7)	.29
E:E' septal	0 (referent)	0.3 (-1.4,2.0)	.73	0.3 (-1.8,2.4)	.80

Values are unstandardized β-regression coefficients (95% CI) from multivariable models, adjusted for sex and gestational age. LBF: low body fat percent; HBF: high body fat percent; MAPSE, mitral annular peak systolic excursion: TAPSE, tricuspid peak annular systolic excursion.

Supplementary 5. Cardiac structure and function across body fat percentiles

	Control (>25th to ≤75th BF%)		LBF (≤10th BF%)		*P* value (LBF vs control)	HBF (>90th BF%)		*P* value (HBF vs control)
Indices of cardiac structure								
	N		**N**			**N**		
PDA present n, %	48	0 (0)	23	2 (8.7)	.14[a]	21	1 (4.8)	
VSD present n, %	48	1 (2.1)	23	1 (4.3)	.80[a]	21	1 (4.8)	
Left ventricle								
Base to apex length, mm	36	27.1 (2.2)	20	25.5 (3.7)	.04	19	29.0 (3.7)	.02
Diameter, mm	36	16.5 (1.4)	20	15.7 (2.7)	.26	19	16.8 (2.2)	.56
Sphericity index	35	1.6 (0.2)	19	1.7 (0.3)	.28	18	1.9 (0.7)	.18
Septal wall thickness/BSA, mm	45	18.4 (2.7)	21	21.1 (3.8)	.007	23	18.6 (4.4)	.88
Posterior wall thickness/BSA, mm	45	15.5 (2.8)	22	18.5 (2.9)	.000	22	14.4 (2.6)	.13
End-diastolic dimension/BSA	46	80.3 (8.3)	22	86.9 (8.8)	.005	23	77.7 (7.2)	.18
Relative wall thickness	45	0.42 (.07)	21	0.46 (.07)	.09	22	0.42 (.07)	.63
Right ventricle								
Base to apex length, mm	33	23.4 (2.4)	14	22.4 (2.9)	.25	17	25.3 (3.4)	.02
Diameter, mm	33	13.8 (1.8)	14	13 (1.4)	.18	17	14.2 (1.6)	.40
Sphericity index	33	1.7 (0.2)	14	1.7 (0.2)	.48	16	1.8 (0.2)	.11
Indices of cardiac function								
	N		**N**			**N**		
Systolic function								
Heart rate	46	135 (22)	20	136 (18)	.87	19	142 (23)	.23
Fractional shortening, %	46	34.6 (7.2)	22	34.3 (5.8)	.83	23	35.6 (6.2)	.60
Left ventricular output	46	230 (69)	19	214 (55)	.39	18	203 (61)	.15
Stroke volume, ml/kg	45	1.8 (0.52)	19	1.6 (0.44)	.27	18	1.4 (0.43)	.03
MAPSE, mm	35	7.0 (1.0)	15	6.6 (0.8)	.20	14	7.1 (1.1)	.62
TAPSE, mm	39	8.7 (1.3)	16	8.4 (1.2)	.52	15	9.4 (1.8)	.11
Mitral lateral S', cm/s	30	5.4 (0.7)	15	5.5 (0.9)	.68	10	5.9 (0.8)	.07
Mitral septal S', cm/s	36	4.9 (0.6)	15	4.9 (0.6)	.89	9	4.8 (0.6)	.81

Tricuspid S', cm/s	24	6.2 (0.6)	15	5.9 (0.9)	.21	9	5.9 (0.8)	.30
	Control (>25th to ≤75th BF%)		**LBF (≤10th BF%)**		***P* value (LBF vs control)**	**HBF (>90th BF%)**		***P* value (HBF vs control)**
Diastolic function								
	N		**N**			**N**		
Mitral E wave, cm/s	36	51 ± 11	21	51 ± 13	.95	15	55 (11)	.21
Mitral A wave, cm/s	36	51 ± 10	21	52 ± 13	.71	15	55 (13)	.25
Mitral E/A	37	1.0 ± 0.1	21	1.0 ± 0.1	.45	15	1.0 (0.1)	.53
Tricuspid E wave, cm/s	40	46 ± 10	20	43 ± 12	.31	15	52 (13)	.11
Tricuspid A wave, cm/s	40	52 ± 8	20	52 ± 9	.70	15	57 (11)	.10
Tricuspid E/A	40	0.9 ± 0.1	20	0.8 ± 0.1	.17	15	0.9 (0.1)	.77
IVRT, ms	32	58 ± 8	18	54 ± 9	.12	13	55 (5.4)	.27
Mitral lateral E', cm/s	30	6.8 ± 1.3	15	6.1 ± 1	.08	10	6.8 (1.4)	.98
Mitral septal E', cm/s	36	5.5 ± 1	15	5.2 ± 1	.48	9	6.0 (1.7)	.17
Tricuspid E', cm/s	24	7 ± 1.6	15	5.9 ± 1.1	.02	9	6.7 (1.6)	.54
E/E' lateral	28	7.3 ± 2.5	15	8.2 ± 2.5	.28	10	8.1 (1.8)	.37
E/E' septal	31	9.1 ± 2.6	15	9.5 ± 2.9	.64	8	9.3 (2.1)	.88

Data presented as mean (SD). Frequency data No. (%). Independent t-test between body fat percentile groups. All cardiac structure and function *P* values adjusted for sex and gestational age. [a] Fisher's Exact test

PDA: patent ductus arteriosus; VSD: ventricular septal defect; BSA: body surface area. MAPSE: Mitral annular plane systolic excursion; TAPSE: Tricuspid annular plane systolic excursion; IVRT: Isovolumetric relaxation time.

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CHAPTER SIX

CARDIAC AUTONOMIC FUNCTION IN THE LATE PRETERM NEWBORN

Original Article

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ABSTRACT

Background: Late preterm newborns make up approximately 74% of all preterm births and 8% of total births. There is an increasing body of evidence demonstrating that babies born late preterm are at an increased risk of neonatal morbidity and mortality. The autonomic nervous system is central in controlling heart rate, effecting rapid changes in heart rate response to stimuli. Such changes enable the newborn to response adequately to changes in the internal and external environment.

Aim: The aim of this study is to investigate whether changes in cardiac autonomic function measured by heart rate variability were present in the late preterm newborn.

Methods: This study included 26 late preterm newborns at 34 and 37 completed week's gestation and a control group of 114 newborns at 37-42 week's gestation. Heart rate variability was assessed in 4-minute epochs repeated up to three times, using time and frequency analysis.

Results: Heart rate variability (total power) was approximately 39% lower in those born late preterm ($P = 0.003$), similarly cardiac parasympathetic activity was approximately 51% lower in those born late preterm ($P = 0.02$). The LF to HF ratio was higher in late preterm newborn compared to full term newborns ($P = 0.04$). Heart rate and low frequency component of heart rate variability did not differ between groups, $P > 0.05$. These associations were independent of newborn sex, birth weight, body fatness and maternal pre-pregnancy body mass index.

Conclusion: Cardiac autonomic control is altered in late preterm newborns compared to term born newborns. This reduced autonomic control during the neonatal period may contribute to increased neonatal morbidity and mortality in late preterm newborns, in addition to potentially predisposing these individuals to higher risk of cardiovascular disease in adulthood.

INTRODUCTION

Eleven percent of all births worldwide are preterm[1]. Approximately, 74% of these preterm infants are born 'late preterm', after 34 weeks gestation, comprising 6-8% of all livebirths [2, 3]. Those born late preterm are often managed during the neonatal phase in the same way as term infants, because they may be appropriate size at birth and appear mature [4]. However, late preterm infants are at a greater risk of medical complications compared to their full-term counterparts[5, 6], including neonatal morbidity and mortality[2, 7, 8] and negative neurologic sequelae [9-11]. Fetal brain growth and development is highest in the last 6 weeks of gestation. At 34 weeks, brain weight is only 65% of that of a brain at 40 weeks [4], Furthermore, between, 34 and 40 weeks gestation cortical volume increases by 50%, and 25% of cerebellar development occurs during this period.

Those born preterm are at increased risk of Sudden infant Death Syndrome during infancy as well as developing high blood pressure as adults [12]. The exact cause of this is unknown; however it is thought that impaired maturation of the autonomic nervous system either via increased sympathetic activity or reduced sympathetic and/or parasympathetic activity may be implicated. Heart rate variability is a widely used non-invasive tool for evaluating cardiac autonomic function. Heart rate variability represents the variation between consecutive heart beat modified by the sympathetic and parasympathetic nervous system. Measures of heart rate variability reflects the hearts ability to respond and adapt to ongoing changes from the internal and external environment [13].

Previous studies in those born preterm show reduced cardiac autonomic activity, specifically a reduction in cardiac parasympathetic activity from infancy to adulthood [13-17]. However, the late preterm infant is less well studied. Given that late preterm newborns make up the

fastest growing subset of neonates [4], the identification of altered risk profile in this group would be of considerable public health relevance. Therefore, we sought to determine whether cardiac autonomic control is reduced in late preterm newborns, compared to those born at term.

METHODS

Ethical approval

This study was conducted in accordance with the NHMRC National Statement of Ethical Conduct in Human Research and was approved by the Sydney Local Health District ethics committee and The University of Sydney Human Ethics committee (HRECH/14RPAH/478). Participation was voluntary, and informed written consent was collected from the parents/guardians.

Subjects

Participants were recruited from the postnatal wards and the neonatal unit at Royal Prince Alfred Hospital, Sydney. This was a pre-specified analysis within a larger prospective cohort study that sought to determine the associations of body composition with cardiovascular health in newborns. Eligible subjects were healthy singleton newborns between 34 and 42 completed weeks gestation. Exclusion criteria for this study was major congenital abnormalities and ongoing need for respiratory support in the newborn. We recruited 33 newborns between 34 and 36 completed week's gestation and 189 term newborns between 37 to 41 week's gestation (Figure 1) as determined by first trimester ultrasound. The control group consisted of 114 newborns. To ensure equal spread across body fat percentiles for comparisons between the full term and late preterm group. The full-term group was selected so that 10% of the newborns were $\leq 10^{th}$ body fat percentile, 15% between $>10^{th}$ to $\leq 25^{th}$, 25%

between >25th to ≤50th, 25% between >50th to ≤75th, 15% between >75th to ≤90th and 10% of newborns >90th percentile body fat percentile. Individuals within body fat percentiles were chosen at random (Figure 1).

Data collection

Demographic data on mothers and newborns were collected directly from mothers using a standardised questionnaire and mother and newborn medical records. We collected current and previous health status, pregnancy and delivery details, and smoking and other lifestyle information.

Body composition and anthropometry

Body composition was measured in newborns in the first 24 hours of life with air-displacement plethysmography (PEA POD®, COSMED, CA, USA, Inc), as part of routine clinical practice. Air-displacement plethysmography is an age-appropriate method for assessing body composition [18], and has been validated in both term and preterm infants [19-21]. This technique accurately measures body volume by the application of Boyle's law to the displacement of air by the infants in a sealed chamber. Fat mass and fat-free mass are calculated by proprietary algorithms. Anthropometry was measured concurrently by two trained midwives. Weight is measured with the integrated PEA POD® scales to the nearest gram, and head circumference to 0.1cm. Length is measured with a length board to the nearest 0.1cm (Easy-Glide Bearing infantometer, Perspective Enterprises, USA). Newborns were categorized according to published gender and gestational age specific body fat percentiles [22].

Heart rate variability

An electrocardiogram (ECG) was recorded continuously for 15 minutes using standard neonatal 3-lead configuration whilst the newborns were sleeping in a supine position. The ECG analogue output was digitised at 500 Hz, and acquired using commercial hardware (Powerlab, ADInstruments, Sydney, Australia). Newborn behaviour was observed closely. Any periods of activity where the newborn woke up were noted and these periods removed from subsequent analysis. Analysis of heart rate and HRV were performed using LabChart (HRV 1 module, version 7, ADInstruments, Sydney, Australia) on up to 3 R-R interval epochs of exactly 4 minutes. Peak detection on ECG was used to create RR sequences. Time domain measures of HRV included the standard deviation of the normal-to-normal (NN) RR intervals as a measure of overall variability, and two short term measures: standard deviation of change in successive NN intervals (SDΔNN), and the root mean square of successive differences in NN interval (RMSSD) [23]. Frequency domain analysis was done by performing a fast Fourier transformation on the RR interval waveform (256 point, Hanning window) with 50% overlap. This provided a resolution (bin width) of 10 Hz. The spectral bands for HRV were investigated in the range of 0-1.1 Hz based on previous studies [12, 24]. Low Frequency at 0.04 to 0.15 Hz, and high frequency at 0.15 to 1.1 Hz. The high frequency band was based on respiratory rates in newborns at 0.5 to 1 Hz.[25, 26] VLF was not determined due to the short sampling times.

Statistical analysis

Statistical analysis was performed using SPSS (IBM Corp, Armonk, NY version 23). Data are expressed as mean and standard deviation (SD) or number and percentage (%). Data were tested for normality by plotting histograms. Log transformations were applied to non-normally distributed data, and normality of the transformed variable was confirmed visually.

Between-group comparisons were performed with independent-samples T-test for continuous data, and Chi-Square or Fisher's Exact tests were utilised for categorical data. Multivariable linear regressions were utilised to compare whether associations were independent of known confounders.

RESULTS

Patient characteristics

Maternal and newborn characteristics are show in Table 1. Mothers in the preterm group were more likely to have a higher pre-pregnancy body mass index compared to mothers in the term group. Maternal characteristics and health were otherwise comparable between groups. Prevalence of maternal smoking and steroid exposure did not differ significantly between groups (Table 1). As expected late preterm newborns were lighter, shorter, had reduced head circumference, body fat and reduced fat free mass compared to full term newborns, Table 1. Rates of admission to the Neonatal Intensive Care (NICU) were not different between groups, highlighting that our preterm group was a healthy group, with the majority managed on the postnatal ward.

Heart rate variability

Heart rate was similar between groups, Table 2. Measures of overall HRV both in the time (SDNN) and frequency domain (total power) were lower in late preterm newborns, being approximately 39% lower for total power. Short-term time domain measures SDΔNN and RMSSD were lower in late preterm compared to full term newborns. For frequency domain measures, there was no difference in LF power between late preterm and term born newborns, but HF power was 51% lower in in the late preterm group, Table 2. Furthermore, the LF:HF ratio was higher in late preterm newborns compared to those born full term.

These associations were independent of newborn sex, body fatness, birth weight and maternal pre-pregnancy BMI (data not shown).

DISCUSSION

Our findings indicate that newborns born late preterm show reduced cardiac autonomic control. Infants born late-preterm often are the same weight and size as those born at term. However, they are physiologically and metabolically immature[27] and may be at an increased risk of developing medical conditions in the immediate postnatal life. To date there have been no studies that have investigated autonomic control in the late preterm newborn, however studies in infants and children born preterm (28 to 37 weeks' gestation) show reduced HRV, as well as reduced parasympathetic modulation to the heart [13-17, 28]. In our study, newborns born late preterm had lower overall HRV (total power and SDNN) and lower parasympathetic modulation of the heart indicated by reduced HF power, RMSSD, SDΔNN, all markers of parasympathetic modulation of the heart. Furthermore, in this study, late preterm newborns showed an increased low frequency:high frequency ratio, thought to be a marker of cardiac sympathetic drive.

These cardiac autonomic changes seen in the late preterm newborn may be exacerbated by maternal and intrauterine exposures. This study did not look to elucidate mechanistic pathways linking late prematurity with cardiac autonomic control. However, we hypothesise that these changes may arise due to impaired development of autonomic regulatory systems even in the late preterm newborn. Studies indicate fetal brain growth and development is highest during the last 6 weeks of gestation. It is possible key brain regions associated with blood pressure control may therefore be impaired, leading to altered cardiac autonomic control[4].

The autonomic nervous system rapidly changes during the last trimester, these changes ensure that the newborn is able to breath, regulate body temperature and obtain food. These progressive changes are critical for normal physiological and behavioural state of the newborn [29]. Furthermore, myelination of the vagus nerve increases significantly between 33-35 weeks' gestation [30]. The vagus nerve is the principal mediator of parasympathetic outflow to the heart, and is central to effecting rapid changes in heart rate in response to stimuli [15].

The reduced overall HRV and parasympathetic modulation to the heart in these late preterm may indicate an impaired inability to adapt to ongoing changes in the internal and external environment. This compromised state obligates the preterm newborn to rely on the sympathetic nervous system to increase heart rate in response to stimuli rather than a vagal withdrawal. This compromised profile may contribute to altered blood pressure control in the immediate postnatal period as well as the long-term control of blood pressure. Indeed, a number of studies show altered blood pressure control in preterm infants [12] and elevated blood pressure in children and adults born preterm [31, 32], it is possible these adverse cardiovascular outcomes may extend to those born late preterm.

CONCLUSIONS

In this study, newborns born late preterm showed evidence of altered cardiac autonomic control, specifically reduced cardiac parasympathetic modulation and increased cardiac sympathetic modulation. The altered cardiac autonomic regulation seen in these late preterm newborns may be associated with increased neonatal morbidity and mortality seen in these newborns, and if these changes persist long-term, may potentially confer increased risk of raised blood pressure in later life.

Figure 1: study flow diagram

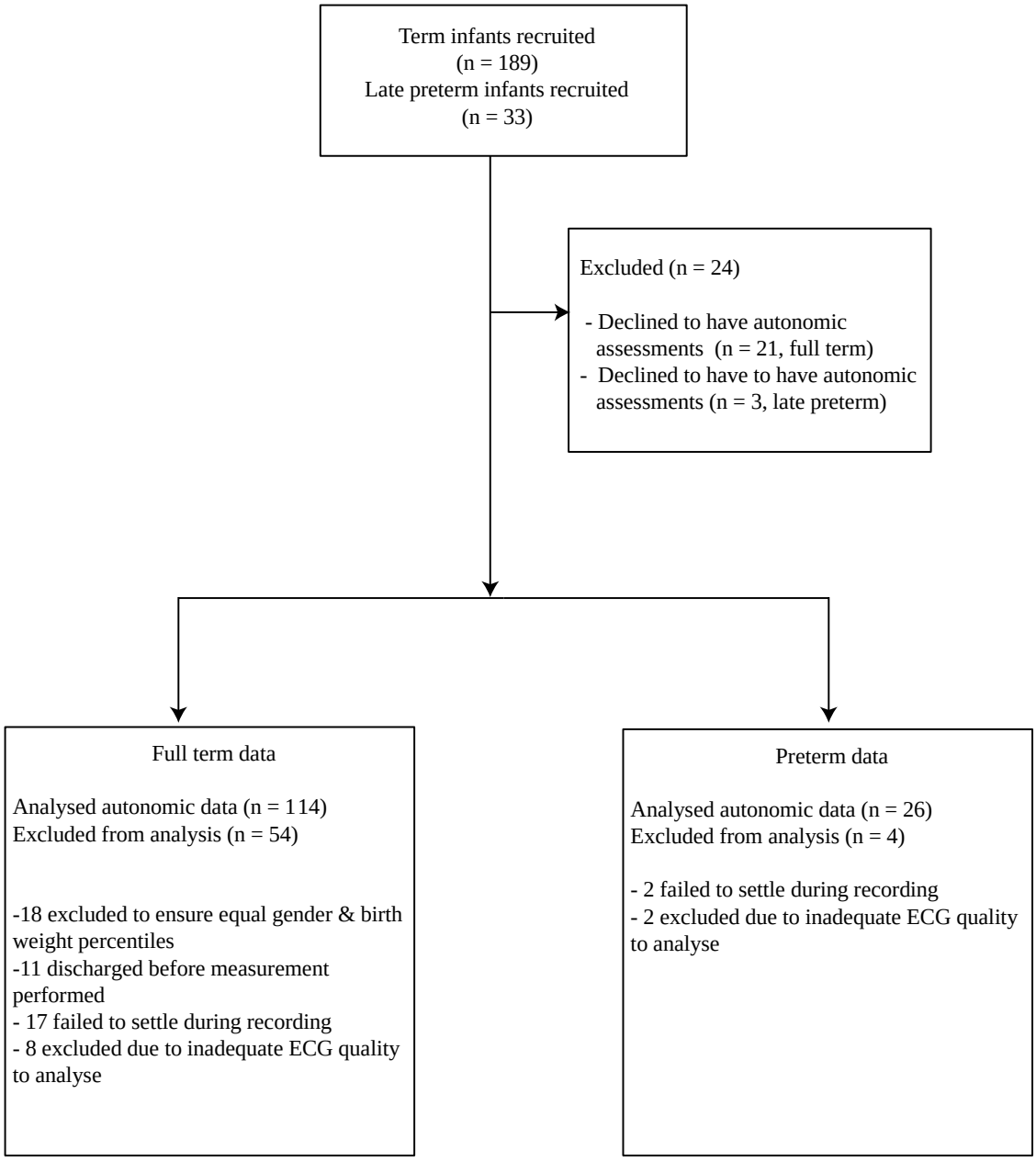

Table 1: Maternal and newborn characteristics

	Full term N = 114	Late preterm N = 26	P value
Maternal Characteristics			
Age, years	33.2 ± 4.6	34.6 ± 5.0	0.17
Ethnicity, n (%)			
Asian	19 (17)	10 (39)	
Caucasian	74 (66)	12 (46)	
Middle Eastern	4 (1)	0 (0)	0.15
South Asian	8 (7)	1 (4)	
Other	6 (5)	3 (12)	
Maternal pre-pregnancy BMI, Kg/m^2	23.1 ± 3.7	25.0 ± 6.1	0.05
Gestational Diabetes Mellitus, n (%)	17 (15)	6 (23)	0.38
Preeclampsia, n (%)	5 (4)	1 (4)	1.00
NICU admissions, n (%)	6 (6)	3 (12)	0.37
Steroid intake, n (%)	4 (4)	1 (4)	0.93
Maternal smoking, n (%)	3 (3)	3 (12)	0.14
Hypertension in pregnancy, n (%)	4 (4)	1 (4)	1.00
Mode of delivery, n (%)			
Normal delivery	67 (59)	18 (69)	
Instrumental	20 (18)	6 (23)	0.19
Caesarean	27 (24)	2 (8)	
Labour			
Spontaneous	62 (54)	19 (73)	
Induced	38 (33)	5 (19)	0.22
No Labour	14 (12)	2 (8)	
Newborn Characteristics			
Gestational age, weeks	39.2 ± 1.1	35.8 ± 0.4	< 0.0001
Postnatal age, days	4.5 ± 4.7	4.1 ± 3.2	0.70
Sex (girls/boys)	62/52	15/11	0.76
Birth weight, g	3436.3 ± 502.1	2806.7 ± 319.8	< 0.0001
Length, cm	49.8 ± 2.3	47.0 ± 2.0	< 0.0001
Head circumference, cm	34.8 ± 33.1	33. 1 ± 1.1	< 0.0001
Body fat, %	11.0 ± 5.1	9.3 ± 3.1	0.03
Body fatness, g	422.0 ± 358.6	259.1 ± 103.8	< 0.0001
Fat free mass, %	89.0 ± 5.1	90.7 ± 3.1	0.10
Fat free mass, g	3017.0 ± 401.7	2548.5 ± 282.6	< 0.0001

Data are presented as mean ± SD for continuous variables using independent t-tests and n (%) for categorical variables, using chi-square tests between full term and late preterm groups. NICU; neonatal intensive care unit. Full term n = 114, late preterm n = 26 except maternal BMI n = 102 (full term).

Table 2. Frequency and time domain measures of heart rate variability between full term and late preterm newborns

	Full term (n = 114)	Late preterm (n = 26)	*P* value (control vs late preterm)
Frequency domain			
*Total Power, ms^2	1051.7 (1114.3)	661.2 (606.5)	0.003
*LF, ms^2	2660.0 (279.7)	232.8 (187.1)	0.43
*HF, ms^2	135.9 (174.0)	82.2 (93.3)	0.02
*LF: HF	2.3 (2.2)	3.1 (2.7)	0.04
Time domain			
HR, bpm	127.5 ± 16.6	132.9 ± 12.2	0.12
Mean NN, ms	477.6 ± 55.8	455.0 ± 42.1	0.06
SDNN, ms	38.0 ± 12.1	29.5 ± 10.4	0.001
*SDΔNN, ms	16.3 (13.1)	12.4 (7.5)	0.01
RMSSD, ms	18.7 ± 10.2	13.4 ± 6.1	0.01

Data presented as mean (SD) or *geometric mean (IQR) for log transformed data. Independent t-test between groups. LF, low frequency; HF, high frequency; LF: HF, low frequency/high frequency ratio; HR; heart rate; SDNN, the mean of the standard deviation of all normal RR intervals; RMSSD, square root of the mean squared differences of successive NN intervals.

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CHAPTER SEVEN

BODY COMPOSITION AND AUTONOMIC FUNCTION IN CHILDREN AND ADOLESCENTS AT REST AND IN RESPONSE TO A STRESS TEST

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ABSTRACT

Background: Over the past four-decade childhood and adolescent obesity has increased by tenfold, a major contributor to increased cardiovascular risk. The autonomic nervous system has been implicated in the pathophysiology of obesity showing an association between increased adiposity and altered autonomic function.

Aim/Methods: The aim of this study was to investigate the association between body composition and autonomic function in children and adolescents 2 to 20 years of age (n = 72) at rest and in response to an exercise test. Body composition was assessed by air displacement plethysomography using the BOD POD (COSMED). Markers of autonomic function include, heart rate variability (HRV), systolic blood pressure variability (SBPV), baroreflex function and dp/dt_{max} variability measured for 5 minutes during rest and 4 minutes during moderate exercise (70% of age predicted heart rate maximum) on a cycle ergometer.

Results: Body fat and fat free mass was not associated with markers autonomic function at rest ($P > 0.05$). However, some associations found after adjustment for the interaction between age and body fatness as well as age and fat free mass. A positive association between body fatness (%) and high frequency component of SBPV was dependent on the interaction between age and body fatness (P interaction < 0.001). In addition, a positive association between fat free mass and heart rate and HRV was dependent on the interaction between age and body fatness (*P* interaction < 0.0001, *P* interaction = 0.01, respectively). There was a significant difference between rest and exercise for all markers of autonomic function ($P < 0.001$ for all). Furthermore, there was a significant association between body fatness, fat free mass and HRV during exercise, (total power, 0.063 [95% CI 0.002, 0.124], P = 0.04), (total power, -0.123 [95% CI -0.213, -0.033, P = 0.01, respectively). The change in

HRV from resting to exercise was associated with fat free mass (total power, -0.165 [95% CI -0.279, -0.051], P = 0.006) which was unaffected after adjusting for resting HRV (total power, -0.124 [95% CI -0.219, -0.029], P = 0.013). Fat free mass was not associated with other markers of autonomic function during exercise (P > 0.05 for all).

Conclusions: At rest, the association between body fat percent and high frequency SBPV was significantly modified by age such that the association was strongest among older participants. Investigating the associations between fat free mass and autonomic function revealed that leaner subjects showed increased HRV, increased cardiac parasympathetic drive and lower heart rates, these associations were strongest among younger participants, 2-9 years of age.

During exercise, individuals with higher body fatness showed increased HRV whereas those with higher fat free mass had lower heart rate variability during exercise. The change in heart rate variability from resting to exercise was only associated with fat free mass, such that a higher fat free mass was associated with a lower change in HRV. This may indicate that those with higher fat free mass (lean body mass) show a reduced response to exercise measured by heart rate variability.

These results are somewhat in line with previous studies indicating that leaner subjects have favourable autonomic function and are at reduced risk or obesity related diseases.

INTRODUCTION

Childhood and adolescent obesity is a major public health concern worldwide. Over the last four decade obesity among children and adolescents has increased by 10-fold [1]. The increasing prevalence of childhood and adolescent obesity is a major concern as children with obesity are more likely to have obesity as adults, increasing their risk of developing cardiovascular disease [2]. Children and adolescents today, compared to those in the last two decade have a higher predicted risk of heart disease in adulthood [3].

Altered autonomic function is implicated as a potential mechanistic link between obesity and cardiovascular disease. The majority of studies in children and adults indicate that obesity alters the cardiac sympatho-vagal balance towards an increased sympathetic modulation and reduced parasympathetic modulation [2]. Analysis of blood pressure variability provides information on vascular autonomic control and an assessment of end-organ autonomic activity [4-6]. Studies in adults show altered vascular autonomic control consistent with an increased sympathetic modulation to the vasculature [6]. Impaired baroreceptor reflex function has also been associated with obesity [7-9]. In addition, studies show altered stress reactivity in obesity [6].

Cardiovascular disease is becoming more prevalent among children and adolescents as rates of obesity increase [10]. A number of studies show obesity is often accompanied by concurrent cardiovascular and metabolic abnormalities. Therefore, immediate attention to understand the underlying mechanistic pathways and prevent cardiovascular damage in children and adolescents is a priority [11-15]. Utilizing body composition measures comprising lean and fat mass may better identify those at increased risk. Accordingly, we sought to determine whether baroreflex function, dP/dt_{max} variability (as a possible new

measure of autonomic regulation of the myocardium), and cardiac and vascular autonomic functions are associated with lean and fat mass in children and adolescents at rest. Furthermore, we sought to determine how these autonomic indices change in response to an exercise test with altered body composition.

METHODS

Ethical Approval

This study was conducted in accordance with the standards set by the 2013 version of the declaration of Helsinki, the National Statement on Ethical Conduct in Human Research 2007 and was approved by the Sydney Local Health district ethics committee (HREC/16/RPAH/80).

Subject selection

Children and adolescents aged 2-20 years of age were recruited via various media including flyers, local newspapers, school newsletters, and The University of Sydney and Royal Prince Alfred Hospital intranet and newsletters. Including/exclusion criteria for the study were that children must be aged 2 – 20 years of age to be included in the study with no specific exclusion criteria. All investigation was undertaken as part of a single visit to the Charles Perkins Centre Royal Prince Alfred Clinic. We recruited a total of 72 children and adolescents for this study, Figure 1.

Body composition and anthropometry

The BOD POD (COSMED, Concord, CA, USA), is a validated non-invasive device that measures body fat percentage and fat free mass in children and adults [16, 17]. This technique accurately measures body volume by the application of Boyle's law to the

displacement of air by the child or adolescent in a sealed chamber, which together with body weight, is used to determine body density. Validated proprietary algorithms are then used to calculate fat mass and fat free mass. Body composition measurements were carried out in a temperature-controlled room (23-25° C). Participants wore minimal, form-fitting clothing and a swim cap to reduce air pockets during the measurement.

Birth weight, length, and head circumference were collected from the participants' 'My Personal Health Record' (the Blue Book) or similar.

Demographics and medical history

Participant characteristics, maternal perinatal details and caregiver characteristics were collected using standardised questionnaires. Information on pregnancy complications was collected from the participants 'My Personal Health Record' (the Blue Book) or similar.

Continuous heart rate and blood pressure measurement

Continuous blood pressure waveform was measured with the human non-invasive blood pressure system (ADInstruments, Sydney, Australia). An appropriate size blood pressure cuff was placed around the middle phalanx of right index finger of the subject. Electrodes were placed for measurement of heart rate via a 3-lead electrocardiogram. A respiratory belt transducer (ADInstruments, Sydney, Australia) containing a piezo electric device was placed around the chest of the subject to measure respiratory rate. The blood pressure, ECG and respiration analogue outputs were digitised at 500 Hz and acquired (Powerlab, ADInstruments, Sydney, Australia). Blood pressure, ECG and respiration were measured during two phases: phase 1 (resting), and phase 2 (exercise test). Participants who did not comfortably reach the foot pedals of the cycle ergometer did not take part in the exercise test, generally children 9 years and below.

Resting phase - phase 1. Participants rested quietly in a supine position for 5 minutes prior to ECG recording. To maintain resting behaviour and reduce the likelihood of younger children moving, a standardized cartoon was offered. Autonomic function analysis was performed over a 5-minute period in each participant during the resting phase.

Exercise test – phase 2. Exercise measurements were made during moderate exercise on a cycle ergometer (Corival cpet, Lode B.V, Groningen, Netherlands). Moderate exercise was defined as 70% of maximum heart rate. Participant maximum heart rate was determined by age-predicted maximum heart rate (maximum heart rate = 220 – age). Maintaining 60-70 rpm on the ergometer, the work load was gradually increased till the participant reached their 70% age predicted heart rate maximum. The participant then continued to maintain this heart rate for a total of 4 minutes. Autonomic function measures were derived from this 4 minute period. Typically, the entire recording period for the exercise test took 8-10 minutes, Figure 2.

Baroreflex function analysis

Spontaneous baroreflex sensitivity (sBRS) was determined using the sequence method which incorporates the identification of sequences of consecutive increases in SBP (pressor ramps) or decreases in SBP (depressor ramps) that are followed by a progressive lengthening (or shortening) of pulse interval (PI) [18]. Spontaneously occurring changes in SBP over a period of 4 or more beats were identified and the relationship with the corresponding pulse interval, with zero delay were plotted. The slope and r^2 value of the linear regression for these plots were calculated and a baroreceptor mediated change in heart rate was only considered to have occurred when the slope was positive and r^2 was >0.8 for each delay.

In addition to sBRS, baroreflex effectiveness index (BEI) was used as an additional measure of baroreflex function in the newborn. This is a measure of barorflex recruitment and was

determined as the ratio of the number of identified baroreflex sequences against the total number of SBP ramps observed for a given period of time [19], calculated using the formula below:

$$BEI = \frac{total\ number\ of\ pulse\ interval/SBP\ sequences}{total\ numer\ of\ SBP\ ramps}$$

Systolic blood pressure variability

Power spectral analysis of the systolic blood pressure (SBP) waveform provides a non-invasive method for the analysis of autonomic nervous system modulation to vasculature. SBPV was calculated using frequency domain methodology on the SBP waveform sampled at 5 Hz, with linear trend removal and by performing a Fast Fourier transform (FFT; 512 point, Hanning window, zero percent overlap) using customised algorithms in Spike2. The FFT was performed on SBP waveform duration of 5 minutes during *phase 1* and 4 minutes during *phase 2* in each participant. Spectral bands of SBPV during *phase 1 (resting)* were defined at 0.04 to 0.15 Hz for low frequency and 0.15 to 0.5 Hz for high frequency. Spectral bands of SBPV during *phase 2 (exercise testing)* were defined at 0.04 to 0.15 Hz for low frequency and 0.15 to 1.1 Hz for high frequency. The very low frequency band was not analysed because of the short time period of the recordings. The high frequency band was based on respiratory rates of the participants during resting and exercise. The total frequency band was defined as the range between 0 to 1.1 Hz.

Heart rate variability

Time and frequency domain analysis of heart rate variability (HRV) were performed using LabChart (HRV 1 module, version 8, AD Instruments, Sydney, Australia). Analysis was performed over 5 minutes during *phase 1* and 4 minutes during *phase 2*.

The Spectral bands of HRV during *phase 1 (resting)* were defined at 0.04 to 0.15 Hz for low frequency and 0.15 to 0.5 Hz for high frequency. Spectral bands of HPV during *phase 2 (exercise testing)* were defined at 0.04 to 0.15 Hz for low frequency and 0.15 to 1.1 Hz for high frequency. The very low frequency band was not analysed because of the short time period of the recordings. The high frequency band was based on respiratory rates of the participants during resting and exercise. The total frequency band was defined as the range between 0 to 1.1 Hz [20].

Time domain measures of HRV included the standard deviation of the normal-to-normal (NN) RR intervals as a measure of overall variability (total power), and two short term measures: standard deviation of change in successive NN intervals (SDΔNN), and the root mean square of successive differences in NN interval (RMSSD) [21].

Spectral analysis of dP/dt_{max} variability

Estimated arterial dP/dt_{max} has been reported to be a surrogate measure for evaluating changes in left ventricular contractility [22], and dP/dt_{max} variability may represent autonomic modulation of ventricular myocardial contractility. The arterial pressure dP/dt is determined by the first differential of the blood pressure waveform, and dP/dt_{max}, the peak rise in blood pressure was identified using a peak detection algorithm in Spike2. Frequency analysis of dP/dt_{max} variability was then performed similar to SBPV. The dP/dt_{max} waveform was resampled at 5 Hz, and linear trend removal and FFT (512 point, Hanning window) was performed. As the maximum rate of change in arterial pressure is related to the force of

ventricular contraction, the low frequency component of dP/dt_{max} variability may reflect the sympathetic modulation of the myocardium during systole.

Statistical analysis

Statistical analysis was performed using SPSS Statistics, version 23 (IBM Corp, Armonk, N.Y., USA). Continuous data were expressed as mean (standard deviation), and categorical data as count (percentage). Data were visually inspected for normality, and non-normally distributed data were transformed appropriately.

The relationship between body composition and autonomic function at rest and in response to exercise were compared using multivariable linear regression. Models were adjusted for age and gender. All exercise test models were adjusted for resting measures. Interaction terms were used to determine whether the associations of body composition with autonomic function differ by age.

RESULTS

Participant characteristics are shown in Table 1. None of the participants were smokers, whilst 3 participants were regular users of asthma inhalers. Exclusion of these 3 participants did not materially affect results (data not shown), and as such were included in all analyses.

Body composition and autonomic function at rest

There were no associations between body fat percent, fat free mass or fat mass with any markers of autonomic function; heart rate variability (HRV), blood pressure variability (SBPV), baroreflex function or dP/dtmax variability at rest ($P > 0.05$ for all, adjusting for sex and age, Table 2).

The associations of body fat percent with heart rate variability and baroreflex function did not differ by age (*P* interaction > 0.05). However, the association between body fat percent and high frequency SBPV was significantly modified by age (*P* interaction < 0.001), such that there was a positive association amongst older participants, which was not observed in younger participants, Figure 3.

The associations of fat mass with heart rate variability, blood pressure variability, baroreflex function and dp/dt_{max} variability were not modified by age (*P* interaction > 0.05, for all). However, the association between fat mass with and heart rate was significantly modified by age (*P* interaction = 0.02), following effect modification we found no association between the two age groups, Figure 4.

The association between fat free mass with time and frequency domain measures of HRV was significantly modified by age. These include total power (HRV), high frequency (HRV), SDRR and heart rate, (*P* interaction = 0.01, *P* interaction = 0.003, *P* interaction = 0.02, *P* interaction < 0.0001, respectively), such that these associations were strongest among younger participants, which was not observed in older participants, Figure 5.

Autonomic function during rest and during an exercise test

There was a significant difference between rest and exercise for all markers of autonomic function, Table 3. Overall heart rate variability (total power and SDRR), LF, HF, RMSSD, baroreflex sensitivity and baroreflex effectiveness index decreased significantly in response to exercise. However the LF:HF ratio of heart rate variability increased in response to exercise. Conversely, blood pressure variability and dp/dt_{max} variability significantly increased in response to exercise, Table 3.

Body composition and autonomic function during exercise

There was a significant association between body fat percent and heart rate variability (HRV) during exercise, (total power, 0.063 [95% CI 0.002, 0.124], P = 0.04), adjusted for age and gender. However, the change in HRV from resting to exercise was not associated with body fat percent (total power, 0.075 [95% CI -0.004, 0.154], (P = 0.06) and adjusting for HRV at rest did not materially affect this association (total power, 0.062 [95% CI -0.000, 0.125], P = 0.05). Body fat percent was not associated with other markers of autonomic function during exercise (P > 0.05 for all).

Similarly, there was a significant association between fat free mass and overall heart rate variability during exercise, (total power, -0.123 [95% CI -0.213, -0.033], P = 0.01), adjusted for age and gender. Furthermore, the change in HRV from resting to exercise was associated with fat free mass (total power, -0.165 [95% CI -0.279, -0.051], P = 0.006) which was unaffected by adjustment for resting HRV (total power, -0.124 [95% CI -0.219, -0.029], P = 0.013). Fat free mass was not associated with other markers of autonomic function during exercise (P > 0.05 for all).

Table 1: Participant characteristics

Participant characteristics N = 72	Value
Age, y	10 (5)
Weight, kg	37 (18)
Height, cm	140 (25)
Gender, male/female	33/39
BMI, kg/m^2	18 (3)
Body fat, %	20 (8)
Fat free mass, %	80 (8)
Fat mass, kg	8 (6)
Fat free mass, kg	38 (15)
Steroid exposure n, %	3 (4)
Smoking, n (%)	0 (0)
Birth details	**Value**
Birth weight, (g)	3334 (454)
Birth length (cm)	51 (2)
Head circumference, (cm)	34 (1)

Data presented as mean (SD) *N* = 72, except for birth weight (n = 67), birth length (n = 61), head circumference (n = 49), body fat (n = 70), fat mass (n = 70), fat free mass (n = 70).

Table 2: Association of body fatness, fat free mass and fat mass with markers of autonomic function in children and adolescents at rest

	Body fatness (%)		**Fat free mass (kg)**		**Fat mass (kg)**	
	β (95% CI)	*P* value	*β* (95% CI)	*P* value	*β* (95% CI)	*P* value
Respiratory rate, br/min	0.05 (-0.06, 0.15)	0.40	-0.14 (-0.3, -0.03)	0.01	0.9 (0.7, 1.0)	0.20
HRV (frequency domain)	**N = 61**		**N = 61**		**N = 61**	
Ln Total Power	- 0.01 (-0.03, 0.03)	0.70	0.01 (-0.02, 0.04)	0.64	0.003 (-0.05, 0.06)	0.90
Ln LF	- 0.001 (-0.03, 0.03)	1.00	0.02 (-0.02, 0.05)	0.53	0.01 (-0.04, 0.06)	0.69
Ln HF	-0.01 (-0.05, 0.03)	0.59	0.004 (-0.04, 0.04)	0.85	-0.01 (-0.07, 0.06)	0.85
Ln LF: HF	0.01 (-0.1, 0.03)	0.36	0.01 (-0.01, 0.03)	0.29	0.02 (-0.02, 0.05)	0.35
HRV (time domain)	**N = 61**		**N = 61**		**N = 61**	
HR, bpm	0.1 (-0.2, 0.5)	0.62	-0.2 (-0.6, 0.2)	0.31	-0.1 (-0.7,0.5)	0.80
SDRR, ms	-0.2 (-1.3, 0.8)	0.67	0.09 (-1.0,1.2)	0.88	-0.1 (-2.0,1.7)	0.88
Ln RMSSD	-0.003 (-0.02, 0.02)	0.73	-0.001 (-0.02, 0.02)	0.95	-0.002 (-0.04, 0.03)	1.0
SBPV (frequency domain)	**N = 58**		**N = 58**		**N = 58**	
Ln Total Power	-0.01 (-0.03, 0.01)	0.44	0.01 (-0.01, 0.3)	0.40	-0.004 (-0.04, 0.03)	0.83
Ln LF	-0.01 (-0.04, 0.01)	0.36	0.01 (-0.01, 0.04)	0.35	-0.01 (-0.05, 0.03)	0.64
Ln HF	-0.01 (-0.02, 0.03)	0.57	-0.01 (-0.03, 0.02)	0.62	0.03 (-0.01, 0.07)	0.19
Baroreflex function	**N = 58**		**N = 58**		**N = 58**	
sBRS	-0.02 (-0.2, 0.2)	0.84	0.18 (-0.05, 0.40)	0.13	0.08 (-0.2, 0.4)	0.64
Ln BEI	-0.02 (-0.04, 0.01)	0.29	0.02 (-0.01, 0.05)	0.16	-0.01 (-0.05, 0.04)	0.77
dP/dtmax variability	**N = 58**		**N = 58**		**N = 58**	
Ln dP/dtmax	-0.004 (-0.02, 0.01)	0.64	-0.01 (-0.03, 0.01)	0.20	-0.01 (-0.03, 0.02)	0.63
Ln Total power	-0.02 (-0.06, 0.02)	0.25	0.0001 (-0.04, 0.04)	1.00	-0.02 (-0.09, 0.04)	0.54
Ln LF	-0.03 (-0.08, 0.02)	0.19	0.01 (-0.04, 0.05)	0.81	-0.03 (-0.1, 0.04)	0.46

Values are unstandardized β-regression coefficients (95% CI) from multivariable models, adjusted for sex and gestational age. Ln log transformed data. HRV, heart rate variability; LF, low frequency; HF, high frequency; LF: HF, low frequency/high frequency ratio; HR; heart rate, mean NN; mean of N wave to N wave variation normal; SDRR, the mean of the standard deviation of all normal RR intervals; SDΔNN, SD change in NN; RMSSD, square root of the mean squared differences of successive NN intervals; SBPV, blood pressure variability; sBRS, spontaneous baroreflex sensitivity; BEI, baroreflex effectiveness index.

Figure 1: study flow diagram

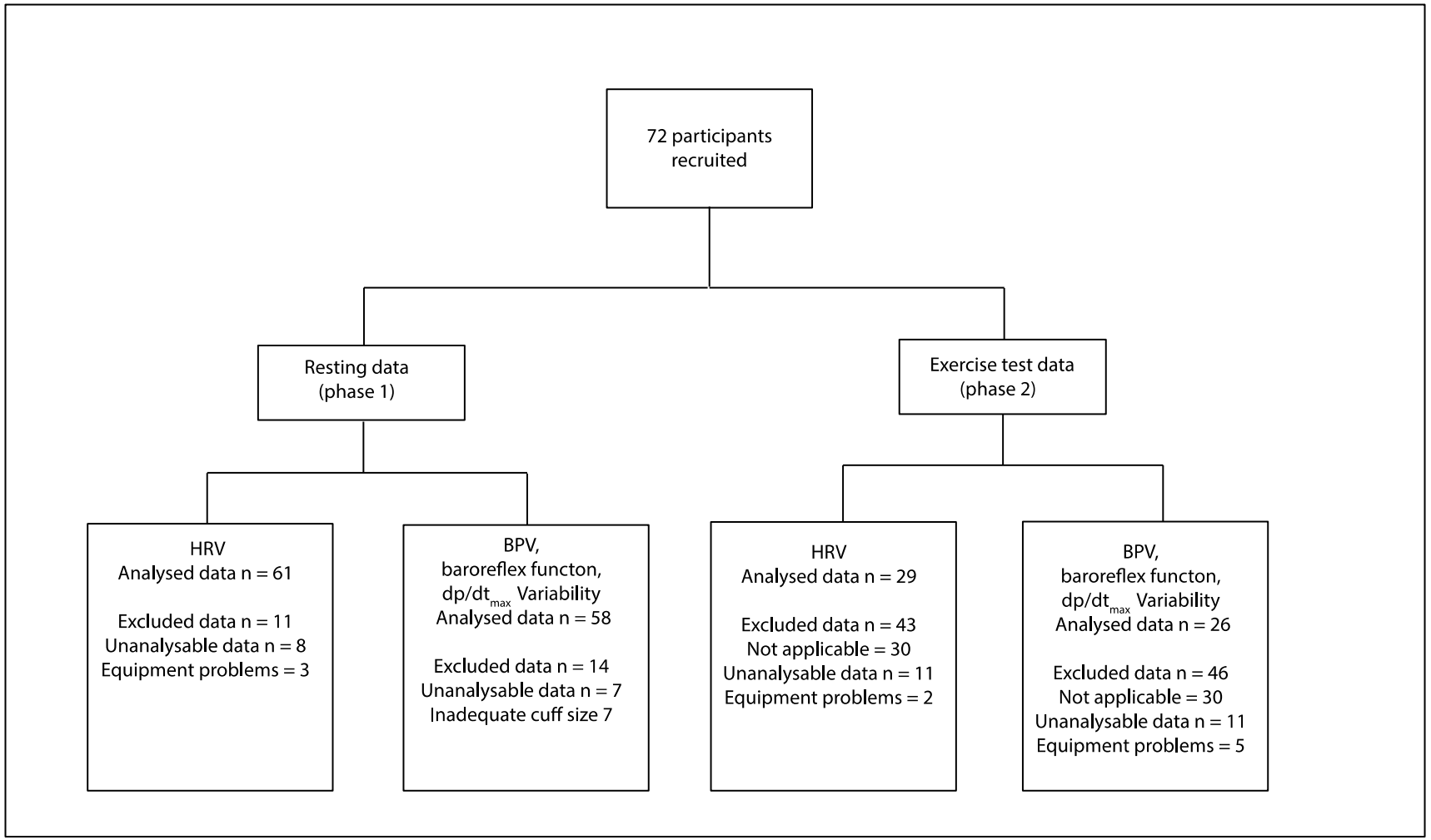
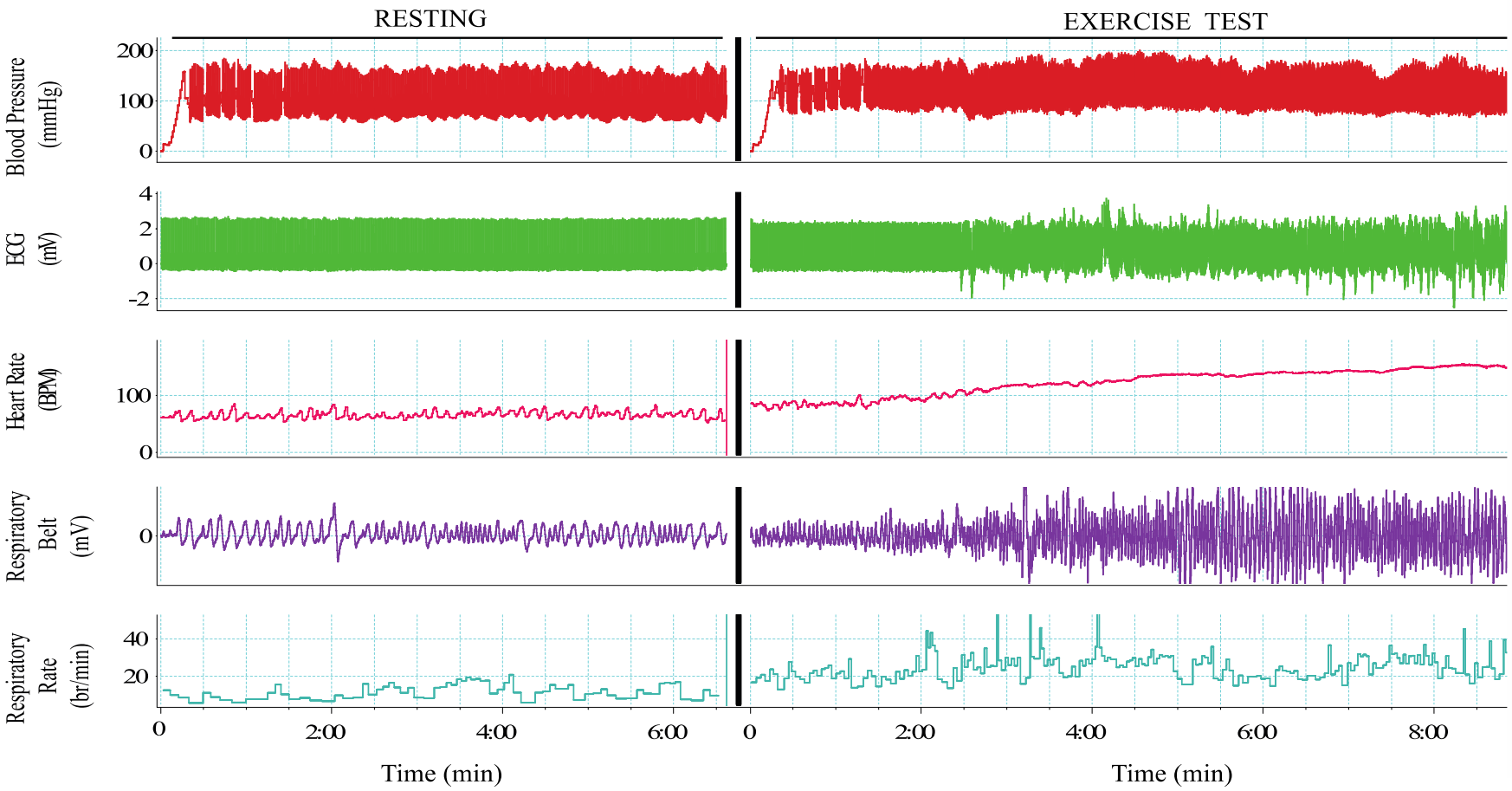


Figure 2: Blood pressure, ECG, respiratory waveform recording and derived parameters during resting and exercise test



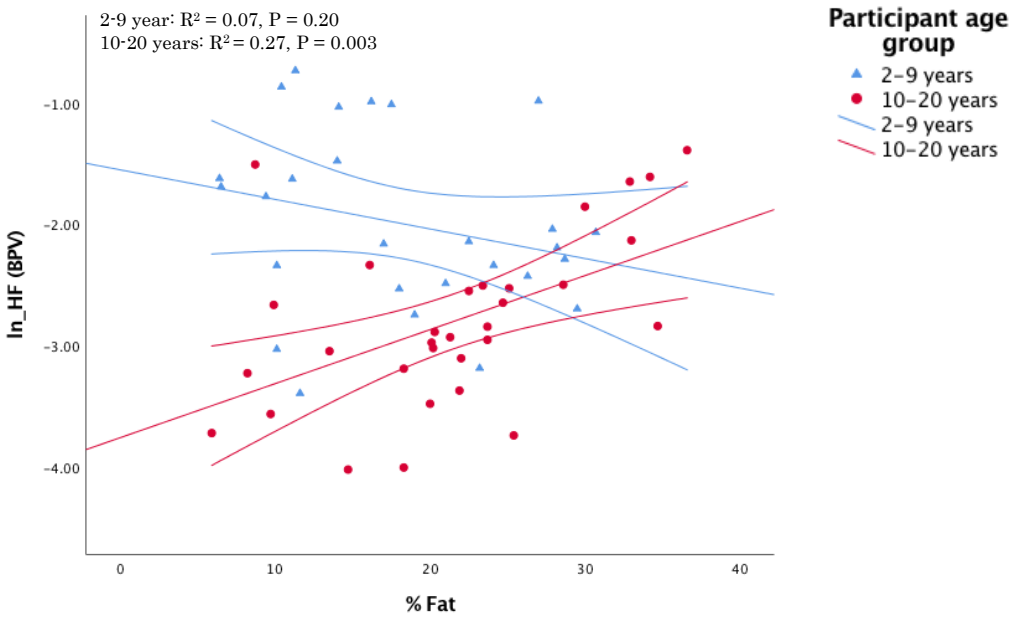


Figure 3: Correlation of body fat percent and ln HF (BPV). HF, high frequency; BPV, blood pressure variability

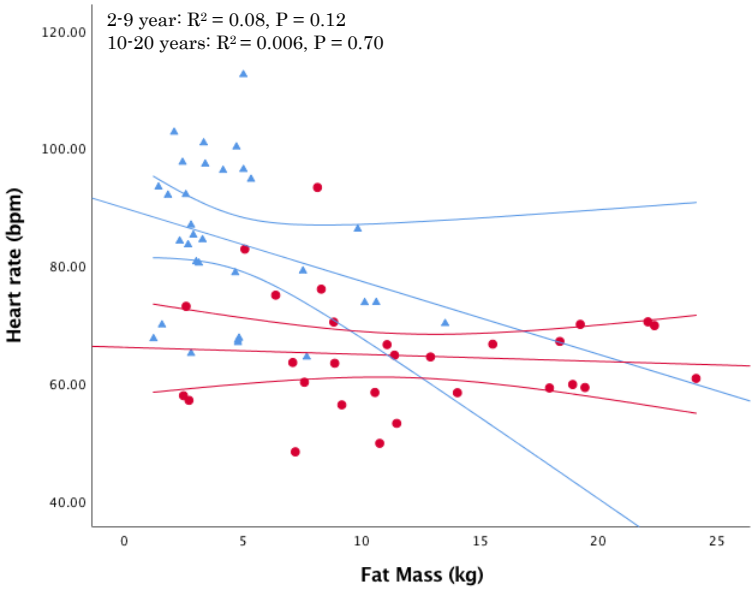


Figure 4: Correlation of fat mass and heart rate.

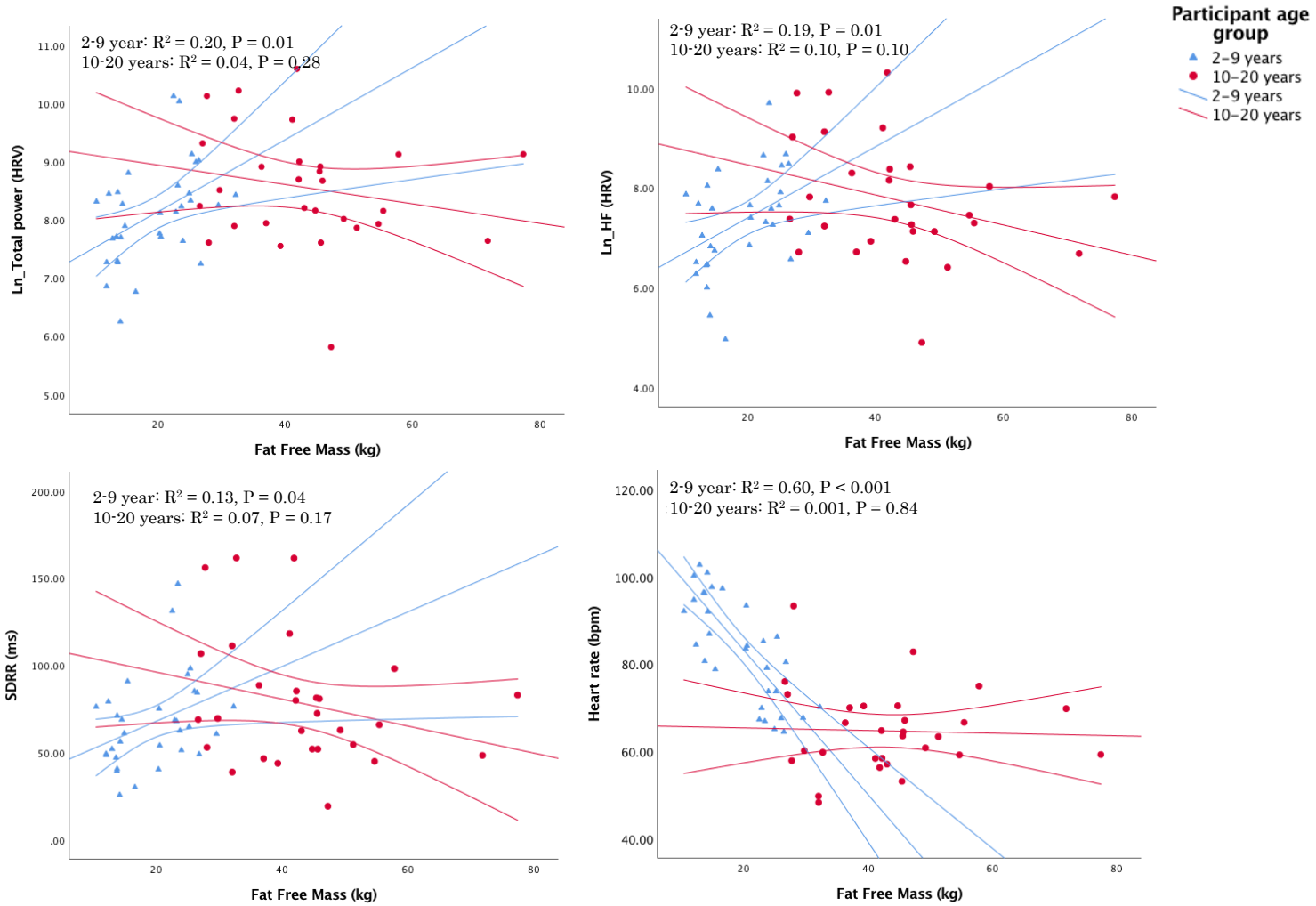


Figure 5: Correlation of fat free mass and ln total power (a), ln HF (b), SDRR (c), heart rate (d)
HRV, heart rate variability; SDRR, standard deviation of RR interval; HF, high frequency

Table 3: Autonomic parameters at rest and at exercise test

	Rest (phase 1)	**Exercise test (phase 2)**	**P value**
Respiratory rate, br/min	17 (4)	29 (6)	<0.0001
HRV (frequency domain)	**N = 29**	**N = 29**	
*Total Power, ms^2	5010 (5412)	449 (1245)	<0.0001
*LF, ms^2	1360 (1662)	76 (129)	<0.0001
*HF, ms^2	2327 (3373)	59 (328)	<0.0001
*LF: HF	0.5 (0.5)	1.3 (2.6)	<0.0001
HRV (time domain)	**N = 26**	**N = 26**	
HR, bpm	65 (8)	124 (18)	<0.0001
SDRR, ms	75 (30)	26 (16)	<0.0001
*RMSSD, ms	71 (57)	12 (22)	<0.0001
SBPV (frequency domain)	**N = 26**	**N = 26**	
*Total Power, $mmHg^2$	0.4 (0.5)	1.8 (1.9)	0.002
*LF, $mmHg^2$	0.2 (0.3)	0.6 (0.5)	<0.0001
*HF, $mmHg^2$	0.1 (0.1)	0.7 (0.7)	0.01
*LF: HF	3.2 (7.1)	0.9 (1.0)	< 0.0001
Baroreflex function	**N = 26**	**N = 26**	
sBRS (ms/mmHg)	12.5 (5.0)	*1.8 (1.3)	< 0.0001
*BEI	0.1 (0.1)	0.06 (0.04)	0.03
dP/dtmax variability	**N = 26**	**N = 26**	
dP/dtmax, mmHg/s	1026 (298)	1523 (873)	<0.0001
Total power, $mmHg2/s^2$	109 (114)	694 (832)	<0.0001
LF, $mmHg2/s^2$	39 (69)	186 (286)	<0.0001

Data presented as mean (SD) or *geometric mean (IQR) for log transformed data. Paired t-test between rest and exercise phase. HRV, heart rate variability, LF, low frequency; HF, high frequency; LF: HF, low frequency/high frequency ratio; HR; heart rate; SDRR, the mean of the standard deviation of all normal RR intervals; RMSSD, square root of the mean squared differences of successive NN intervals; SBPV, blood pressure variability; sBRS, spontaneous baroreflex sensitivity; BEI, baroreflex effective index

DISCUSSION

The aim of this study was to investigate the association between body composition and autonomic function in children and adolescents at rest and in response to an exercise test. At rest, the association between body fat percent and high frequency SBPV was significantly modified by age such that the association was strongest among older participants. Investigating the associations between fat free mass and autonomic function revealed that leaner subjects showed increased HRV, increased cardiac parasympathetic drive and lower heart rates, these associations were strongest among younger participants, 2-9 years of age. During exercise, individuals with higher body fatness showed increased HRV whereas those with higher fat free mass had lower heart rate variability during exercise. The change in heart rate variability from resting to exercise was only associated with fat free mass, such that a higher fat free mass was associated with a lower change in HRV. This may indicate that those with higher fat free mass (lean body mass) show a reduced response to exercise measured by heart rate variability.

Obesity among children and adolescents has been increasing over the past four decades and the autonomic nervous system has been implicated in the disease progression [2]. This study found that the association between body fat percent and high frequency SBPV was significantly modified by age such that a positive association was observed among older participants. The high frequency component of SBPV in autonomic regulation is thought to be linked to respiratory modulation of sympathetic vasomotor tone. This rhythmic drive in phase with respiration producing high frequency oscillations in blood pressure are known as Traube-Herine waves and involve intrinsic central coupling between neurons that generate vasomotor and respiratory activities [23]. Studies indicate amplified sympathetic nerve activity as the respiratory frequency contributes to the development rather than the

maintenance of hypertension [24]. Furthermore, studies in humans show amplified Traube-Herine waves in young, normotensive adults at risk of developing hypertension [23]. The exact cause of increased high frequency SBPV in those with high body fat is unclear, however a strong association between increased adiposity and increased sympathetic drive has been shown previously [25]. It is possible that those with increased body fatness as young as 10-20 years of age are already showing altered autonomic function illustrated by increased high frequency SBPV. We found no other associations between body fatness and other markers of autonomic function.

Investigating the associations between fat free mass and autonomic function revealed that leaner subjects showed increased HRV (total power and SDRR), increased high frequency HRV (marker of cardiac parasympathetic drive) and lower heart rates; these associations were only evident among younger participants, 2-9 years of age. HRV reflects the heart's ability to adapt to ongoing changes in the internal and external environment and a lower HRV is a prognostic for adverse cardiovascular events [26, 27]. Our study findings indicate that children who have increased fat free mass (lean subjects) show favourable cardiac autonomic function with increased HRV, increased cardiac parasympathetic drive and lower heart rates.

We found no other associations between body fatness, fat mass or fat free mass and other autonomic function indices.

Autonomic function during exercise

It is possible that a disease phenotype may only be unveiled when individuals are challenged with an acute stressor, such as moderate exercise. To date only a few studies have investigated the pattern of autonomic variables during exercise.

In our study, overall HRV and cardiac parasympathetic activity and baroreflex function was significantly reduced in response to exercise, however the LF : HF ratio of HRV was significantly increased during exercise. The LF : HF ratio is often considered as a marker of autonomic nervous system balance and a higher ratio is thought to be associated with increased cardiac sympathetic balance [27]. Conversely, SBPV and individual frequency components, low and high frequency were significantly increased in response to exercise indicating vascular autonomic control and end organ autonomic function is significantly elevated. Similarly, cardiac contractility measured by dP/dtmax was significantly increased along with dP/dtmax total variability and low frequency dP/dtmax variability.

Body composition and autonomic function during exercise

We further investigated the effect of body composition on markers of autonomic function during exercise. A normal cardiovascular adaptation to exercise in healthy individuals is a parasympathetic withdrawal, resulting in increased heart rate and a pronounced reduction in HRV [28, 29]. In our study individuals with higher body fatness showed increased HRV whereas those with higher fat free mass had lower heart rate variability during exercise. The change in heart rate variability from resting to exercise was only associated with fat free mass, such that a higher fat free mass was associated with a lower change in HRV. This suggests that those with higher body fatness respond to exercise with greater variability in heart rate possibly indicating an exacerbated response to exercise. Conversely, those with increased fat free mass showed reduced HRV, and the change from resting to exercise was lower. This may indicate that those with higher fat free mass (lean body mass) show a reduced response to exercise measured by heart rate variability. These results are somewhat in line with previous studies indicating that leaner subjects possess favourable autonomic

function and are at reduced risk or obesity related diseases. We found no other associations between body fatness and other markers of autonomic function during exercise.

Strengths and Limitations

One of the strengths in our study is that we have successfully recorded and analysed a number of key markers of autonomic function across a broad cross-section of childhood and adolescence, from 2 to 20 years of age. Many studies only assess heart rate variability, which is a measure of cardiac autonomic function alone, and does not represent autonomic activity to other regions including the vasculature. Furthermore, it is increasingly accepted that measures of blood pressure variability and baroreflex function are also associated with disease pathology, therefore, optimising these markers and measuring them in younger individuals is important in the understanding of disease progression.

There are a number of limitations in this study. Firstly, we were unable to obtain exercise stress tests data in all participants, particularly the younger 2-9-year olds, as they were unable to pedal the cycle ergometer safely. Although autonomic function was significantly altered from resting to exercise, not all participants reached their 70% of heart rate max. We believe this is because not all subjects were accustomed to cycling, therefore premature muscular fatigue may have prevented these individuals from reaching their maximal effort. Future studies should look to evaluate individual physical capacity and effort tolerance.

CONCLUSION

At rest, the association between body fat percent and high frequency SBPV was significantly modified by age such that there was a direct association amongst older participants (aged 9-10 years). Investigating the associations between fat free mass and autonomic function revealed that those with higher fat free mass showed increased HRV, increased cardiac

parasympathetic drive and lower heart rates, these associations were strongest among younger participants, 2-9 years of age.

During exercise, individuals with higher body fatness showed increased HRV whereas those with higher fat free mass had lower heart rate variability during exercise. The change in heart rate variability from resting to exercise was only associated with fat free mass, such that a higher fat free mass was associated with a lower change in HRV. This may indicate that those with higher fat free mass (lean body mass) show a reduced response to exercise measured by heart rate variability. This suggests that worsening of autonomic control due to adiposity may develop over time during childhood and adolescence.

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CHAPTER EIGHT

CONCLUSIONS, IMPLICATIONS OF THIS RESEARCH AND FUTURE DIRECTIONS

CONCLUSIONS AND IMPLICATIONS OF THIS RESEARCH

Role of the placenta in fetal growth - neonatal study

In chapter three we assessed associations between maternal factors, placental function and inflammation with newborn body composition. We further examined whether placental weight mediated the association of maternal factors (pre-pregnancy weight and age) with newborn body composition. Over recent years there has been an increasing interest in the role of the placenta and factors which affect birthweight. However, little is known about the factors that control newborn body composition, fat mass and fat free mass, or the effects of newborn body composition on neonatal health parameters. In this study, we found maternal factors that were associated with newborn fat mass and fat free mass differed, suggesting different mechanisms controlling fat mass and fat free mass in the newborn. In addition, this study showed that placental weight and surface area were strongly associated with both fat mass and fat free mass.

Studies indicate that early life inflammatory events may be important in programming later cardiovascular disease, contributing to causal pathways that underpin epidemiological associations between perinatal factors such as poor intrauterine growth. Therefore, this study also sought to determine whether there was an association between an early life inflammatory exposure and body composition. Contrary to our hypothesis we found little evidence for this. However, it is possible the low statistical power of the study to examine these relationships have meant that an effect may have gone undetected. Furthermore, placental weight partly mediated the association of maternal factors with newborn body composition. We believe that this study is the first to assess associations between maternal factors, placental function

and inflammation with newborn body composition and attempt to delineate direct and indirect associations.

Newborn body composition, born late preterm and vasomotor function – neonatal study

In chapter four we describe experiments where we examined the effect of newborn body composition on newborn autonomic control of vasomotor function, specifically, blood pressure variability and baroreflex function. Furthermore, we introduced a possible new non-invasive measure of autonomic regulation of the myocardium, dP/dt_{max} variability of arterial pressure. In this study we found blood pressure variability was similar across body fat percentiles, and between those born at term and those born late preterm. However, baroreflex sensitivity was significantly reduced in those with high body fatness. Furthermore, there was a non-linear association of newborn body fat with baroreflex sensitivity, which was independent of birthweight.

Previous studies in children and adults with obesity have consistently shown a reduced baroreflex sensitivity. Our results extend these findings indicating that reduced baroreflex sensitivity is apparent in the newborn with increased body fatness. To date there are a number of studies that indicate babies born at the extremities of the birthweight spectrum are at increased risk of developing cardiovascular disease, although the mechanisms remain unclear. It is possible that the autonomic nervous system may be implicated in this increased risk. To our knowledge this is the first study that has investigated newborn body composition and vasomotor function in the newborn.

This novel index, dP/dt_{max} variability proposed as a measure of autonomic regulation of the myocardium, may be of value in evaluating paediatric and adult patients with

autonomic dysfunction. Further investigation and validation of dP/dt_{max} variability using specific iontropic and chronotropic agents is important and the subject of future studies.

Newborn body composition and cardiac autonomic function – neonatal study

Birthweight is associated with cardiovascular disease, such that those at both end of the birthweight spectrum are at increased risk. However, birthweight is only a crude marker of fetal growth and may not accurately differentiate between the constitutionally small infant who has met their genetic growth potential from those of the same weight but who are pathologically growth restricted. Similarly, those who are large may have reached their predetermined growth potential or may be disproportionately large. In an attempt to distinguish between these groups of infants we assessed infant body composition, and measured heart rate variability, a widely used tool to assess cardiac autonomic function. We demonstrated that infants with low body fatness have significantly reduced heart rate variability.

Previous studies show altered autonomic function in people born with low birthweight, from infancy thought to adult life. However, our findings are the first to detail associations with body fatness. We also report for the first time that infants with high body fatness have lower overall heart rate variability, consistent with an overall reduction in parasympathetic modulation to the heart. The influence of adiposity on overall heart rate variability and parasympathetic activity in children and adults with increased adiposity is remarkably consistent with our study, showing reduced heart rate variability and vagal withdrawal in these individuals. Furthermore, the association of body fatness with heart rate variability was independent of birthweight and more strongly associated with body fatness than birthweight.

This study suggests that body fatness may provide information beyond that obtained from birthweight assessments alone and may be an important factor in identifying individuals at risk of later cardiovascular disease.

Late preterm newborns and cardiac autonomic function – neonatal study

Findings from this study indicate those born late preterm show reduced overall heart rate variability and reduced parasympathetic modulation to the heart. In addition, late preterm infants showed an increased low frequency: high frequency ratio, thought to be a marker of cardiac sympathetic drive. Previous studies in preterm infants show altered blood pressure control and elevated blood pressure in children and adults born preterm. Our findings indicate altered cardiovascular outcomes extend to those born late preterm.

Body composition and autonomic function – child & adolescent study

At rest, the association between body fat percent and high frequency SBPV was significantly modified by age such that there was a direct association amongst older participants (aged 9- 10 years). Investigating the associations between fat free mass and autonomic function revealed that those with higher fat free mass showed increased HRV, increased cardiac parasympathetic drive and lower heart rates, these associations were strongest among younger participants, 2-9 years of age.

During exercise, individuals with higher body fatness showed increased HRV whereas those with higher fat free mass had lower heart rate variability during exercise. The change in heart rate variability from resting to exercise was only associated with fat free mass, such that a higher fat free mass was associated with a lower change in HRV. This may indicate that those with higher fat free mass (lean body mass) show a reduced response to exercise measured by

heart rate variability. This suggests that worsening of autonomic control due to adiposity may develop over time during childhood and adolescence.

Taken together, the series of studies conducted as part of this thesis has identified groups with altered autonomic function. This thesis also highlights that body composition maybe an important additional predictor of cardiovascular risk.

FUTURE DIRECTIONS

Newborn body composition

We investigated several predictors (maternal and placental factors) of newborn body composition and examined the effects of newborn body composition on a number of noninvasive markers of autonomic function. Following the investigations in this thesis, there are a number of future directions into the research of newborn body composition that should be addressed. Firstly, studies concerning fetal programming should consider neonatal body composition as an additional predictor of nutritional status *in utero* rather than birthweight alone. However, it is important to note that neonatal body fatness is better reflective of nutritional status in the last trimester of pregnancy, and therefore body fatness may identify specific aspects of cardiovascular risk modulated in the last trimester. Identifying individuals at risk of later cardiovascular disease remains a priority. Therefore, to characterize if these changes seen in infancy persist to later life, follow up studies are required.

Using birthweight percentiles and body fat percentiles will allow us to identify newborns at risk of cardiovascular morbidity and mortality in the immediate neonatal period as well as in later life. However, there are infants within the low and high ends of birthweight and body fat percentiles that have autonomic function comparable to those in the normal categories. Perhaps a “risk score” which incorporates several factors including maternal and placental factors may better identify the high risk infant.

The immune system is a key mediator in the development of cardiovascular disease. Early life inflammatory events which affect the placenta may be important in programming later cardiovascular disease. However, only a few studies have investigated the possible

contributory role of prenatal inflammation on cardiovascular risk. Therefore, whether a prenatal inflammatory exposure may contribute to increased cardiovascular risk in later life is yet to be investigated comprehensively.

Noninvasive markers of autonomic function

Over the recent years a significant relationship between the autonomic nervous system and cardiovascular mortality has been recognized with an established relationship between increased sympathetic nerve activity and cardiovascular disease in the adult.

Given that those born at the extreme ends of the birthweight spectrum are at an increased risk of cardiovascular disease, it is important to understand the origins of altered autonomic function beginning in the newborn. To date it is unclear how autonomic function throughout the lifespan may change and modulate risk of later cardiovascular disease. Measures of heart rate variability represents a promising marker of autonomic function and is now used in clinical practice by cardiologists, and as an important research tool. However, heart rate variability is concerned with cardiac autonomic function only and does not discriminate cardiac from global autonomic activity. Autonomic modulation of the vasculature, including the kidney and the baroreflex, may play a crucial role in the development of the disease. The significance of blood pressure variability on cardiovascular risk has also been recently recognized. Future longitudinal and intervention studies incorporating autonomic function measures across the lifecourse may improve risk stratification.

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APPENDIX ONE

AUTONOMIC DYSFUNCTION: A POTENTIAL MECHANISM IN PROGRAMMED HYPERTENSION

Editorial

Dissanayake, H., & Phang, M. (2016). Autonomic Dysfunction: A potential Mechanism in Programmed Hypertension. *J Paediatr Lab Med*, *1*, e101.

The following appendix contains a published editorial discussing autonomic dysfunction in programmed hypertension.

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Dissanayake and Phang, J Paediatr Lab Med 2016, 1:e101

Editorial OMICS International

Autonomic Dysfunction: A potential Mechanism in Programmed Hypertension

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Editorial

Hypertension, a disease of deregulation of blood pressure control, is a major risk factor for cardiovascular and cerebrovascular diseases [1] and is reported to affect approximately 40% of people [2]. In addition to the high prevalence and predicted increases incidence of hypertension, successful treatment remains poor and it has been argued that this is partly due to the poor understanding of causative mechanisms. The autonomic nervous system is central to the control of blood pressure and a neurogenic component of hypertension is becoming increasingly realised as several lines of evidence in humans and animal models show convincingly that abnormalities in autonomic control of blood pressure plays a major role in the etiology of disease and cardiovascular mortality. The mammalian nervous system begins its development during fetal life and continues after birth. Therefore, it is thought that both intrauterine and postnatal insults may sensitise and alter the development of different components of the nervous system.

There is a robust body of evidence indicating that a number of different nutritional manipulations or other adverse variations in the prenatal environment program hypertension; this has been shown convincingly in a number of animal models [3]. Whilst the resultant pathophysiology underlying programmed hypertension is uncertain and may be varied, there is evidence for autonomic dysfunction as a possible causative pathophysiological mechanism underlying the development of fetal programming of hypertension.

To date there has been limited interest in autonomic dysfunction as a potential mechanism in programmed hypertension which may be due to historical reasons as well as technical limitations associated with autonomic measurements.

Historically, the kidney was considered the sole contributor of long term blood pressure control, this theory stems from the teachings of the highly influential Guyton model of blood pressure control. Therefore, many of the work investigating programmed hypertension explored kidney dysfunction as a plausible mechanism in determining the etiology of programmed hypertension. Studies investigating the kidney have indeed shown windows of susceptibility to blood pressure programming that appear to occur during the early stages of kidney development, showing a reduction in glomeruli and nephron number [4-6]. However, there is conflicting evidence as studies show hypertension in the absence of reduced nephron number [7] and reduced nephron number with no apparent hypertension [8]. Although it is physiologically plausible that abnormalities in the kidney may be of importance to the etiology of programmed hypertension, the data indicate that while nephron deficit may play a permissive role, it is not the primary cause of programmed hypertension.

Over the last decade technological advancement have greatly improved our ability to accurately quantify or measure autonomic function. Earlier studies investigating blood pressure of programmed rats used more invasive techniques, such as tail cuff plethysmography. It has been postulated that these types of invasive techniques may be inducing a stress response and therefore the observed increased blood pressures may not be reflective of resting hypertension but that of an exaggerated stress response. Animal models have been used extensively to investigate programmed hypertension using a variety of maternal manipulations [3] however only a few have investigated autonomic dysfunction as a potential mechanism. A study by Samuelsson and colleagues [9] showed hypertension and increased sympathetic activity in rat offspring from mothers fed an obesogenic diet during pregnancy. In addition, hypertension and changes to sympathetic activity was established in the juvenile rate indicating that hypertension and changes in autonomic activity arise as a direct consequence of in utero exposure to a maternal obesogenic diet. Similarly, a study investigating a high fat diet in the absence of maternal obesity in rats showed increased sympathetic activity and blood pressure only in response to a stressor [10]. Studies in rabbits also show increased blood pressure and renal sympathetic nerve activity in offspring from mothers that were fed a high fat diet. Furthermore, an exaggerated sympathetic response was seen when these rabbits were exposed to an acute stressor [11].

Non-invasive measures of autonomic function are imperative when translating these effects into human studies. Recent techniques of heart rate variability and blood pressure variability have facilitated the exploration of autonomic dysfunction as a potential mechanism in programmed hypertension. Several studies of growth-restricted children show changes to cardiovascular control, these include altered blood pressure [12], increased pulse rate [13] and change in heart rate fluctuations [14]. Assessment of heart rate variability has shown that infants born small-for-gestational-age (SGA) exhibit increased sympathetic activity at one and three months of age compared to infants born appropriate-for-gestational age [15]. Beyond childhood, in adults born SGA, direct recording of sympathetic nerve traffic using microneurography showed increased sympathetic nerve activity compared to their control counterparts, who did not differ by central adiposity, body mass index or glucose tolerance; therefore metabolic factors did not attribute to the increased sympathetic activity seen in these SGA individuals [16]. Furthermore, a strong association between low birth weight and a shorter pre ejection period, indicative of a stronger sympathetic control of heart rate [17,18] and increased sympathetic activation of the heart has also been reported [17].

In line with these findings, studies have also shown an association between diminished parasympathetic control and increased risk of malignant cardiac arrhythmias and hypertension [19].

Young adults born with extremely low birth weight exhibit a significantly decreased parasympathetic regulatory capacity compared to their control counterparts during their second and third decade of life [19]. Collectively, these studies suggest that increased sympathetic and reduced parasympathetic activity may manifest during early development, and that autonomic dysfunction is a plausible mechanism underlying the increased risk of hypertension in individuals who had intrauterine growth restriction [16,20].

There is increasing evidence for autonomic dysfunction as a potential mechanism in programmed hypertension. However, to explore the exact mechanism by which this occurs requires further experimental investigation incorporating recent techniques in measuring autonomic function.

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APPENDIX TWO

MATERNAL N-3 FATTY ACIDS AND BLOOD PRESSURE IN CHILDREN

Book Chapter

* Authors contributed equally

Dissanayake, H. U*., Phang, M*., & Skilton, M. R. (2017). Maternal n-3 Fatty Acids and Blood Pressure in Children. In *Diet, Nutrition, and Fetal Programming* (pp. 279-292). Humana Press, Cham.

The flowing appendix contains a published book chapter evaluating evidence linking maternal intake of omega-3 polyunsaturated fatty acids and offspring blood pressure.

Chapter 21
Maternal n-3 Fatty Acids and Blood Pressure in Children

Hasthi U.W. Dissanayake, Melinda Phang, and Michael R. Skilton

Key Points

- The potential for 'deprogramming' of hypertension and the underlying mechanisms are poorly understood.
- Animal studies indicate that long-chain omega-3 polyunsaturated fatty acid insufficiency in the perinatal period is associated with raised blood pressure in later life.
- Overall evidence for maternal omega-3 polyunsaturated fatty acid intake influencing offspring blood pressure in humans is inconclusive.
- Some cohort studies suggest an association between higher maternal omega-3 polyunsaturated fatty acid intake in late pregnancy with lower offspring blood pressure in childhood.
- Post hoc analyses of dietary interventions increasing maternal omega-3 polyunsaturated fatty acid intake during gestation or lactation have shown no effect on offspring blood pressure.
- There is a lack of evidence from prospective randomised trials of maternal omega-3 polyunsaturated fatty acid with offspring blood pressure as a prespecified outcome.
- There is a lack of evidence for associations of maternal omega-3 polyunsaturated fatty acid intake with offspring blood pressure in high-risk populations.

Keywords Omega-3 polyunsaturated fatty acids • Blood pressure • Developmental origins • Pregnancy • Lactation • Fetal programming • Life course

Abbreviations

ALA	Alpha-linolenic acid
BP	Blood pressure
DBP	Diastolic blood pressure
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid

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GA	Gestational age
LCPUFA	Long chain polyunsaturated fatty acids
MAP	Mean arterial pressure
n-3 PUFA	Omega-3 polyunsaturated fatty acids
n-6	Omega-6 polyunsaturated fatty acids
SBP	Systolic blood pressure

Introduction

High blood pressure (BP) is a major risk factor for cardiovascular and cerebrovascular diseases, which in turn are the leading cause of morbidity and mortality worldwide [1]. For each 20 mmHg increase in systolic BP (SBP) or 10 mmHg increase in diastolic BP (DBP) the risk of cardiovascular disease increases by twofold [2].

Physiological control of BP involves the complex, yet precise, interaction between different organs and the continuous actions of the cardiovascular, renal, neural and endocrine systems. Under normal function, BP is tightly regulated, maintaining sufficient pressure to ensure constant perfusion of end organs and tissues, without causing pressure related structural damage [3]. This tight regulation of BP is achieved through combination of local and systemic mechanisms, whereby local mechanisms acutely regulate blood flow via vasoconstriction and dilatation, and global mechanisms act via the autonomic nervous system. The sympathetic branch of the autonomic nervous system affects BP control via direct adjustments to cardiac output and total peripheral resistance, and indirect adjustments to blood volume via changes in renal function. In contrast, the renal endocrine system is a powerful long-term regulator of BP, principally achieved via water and salt excretion related changes in blood volume.

Pathophysiology of Programmed Hypertension

There is now an extensive body of experimental and clinical work demonstrating that the intrauterine environment is a predictor of BP, and describing the underlying mechanisms. Mechanisms implicated include the kidney – with evidence for critical periods of exposure [4]; the vasculature – with extensive evidence that various forms of maternal malnutrition can affect nitric oxide dependent vasodilatation and microvascular density [5], key local mechanisms influencing total peripheral resistance and thus BP; and the autonomic nervous system. A number of studies show increased sympathetic activity [6], decreased parasympathetic activity [7], and changes to autonomic function [8] in children born small for gestation age, a group with at risk of elevated BP from childhood through adulthood [9]. This is supported by experimental models showing autonomic and baroreflex dysfunction induced by a number of maternal nutritional manipulations [10]. Nonetheless, the fine details of these mechanisms driving programmed hypertension remain elusive, although may provide an indication of potential targets for interventions.

Programmed Hypertension: Potential for Prevention or Early Treatment

High BP is one of the most modifiable risk factors and strong evidence supports that it can be affected by pharmacological agents and lifestyle interventions, including dietary strategies [11]. Effective strategies to counter programmed hypertension remain poorly described. Due to potential risks to the

developing fetus and infant, safety is a key consideration for interventions during this period. Accordingly, maternal nutritional interventions are likely a practicable means by which to prevent programmed hypertension, with omega-3 polyunsaturated fatty acids (n-3 PUFA) being one such nutritional hemodynamic agent.

n-3 PUFA, Metabolism and Physiology

Embedded in the phospholipids of cellular membranes, fatty acids play vital biochemical and physiological roles serving as lipid platforms to drive mechanistic and signalling pathways. In particular, the essential PUFAs, namely n-3 α-linolenic acid (ALA; C18:3n-3) and n-6 linoleic acid (C18:2n-6), which cannot be synthesised by mammalian cells and are essential for normal physiological function. ALA is on average the most readily consumed n-3 PUFA, obtained predominantly from plant sources, and can be endogenously converted into longer chain n-3 PUFAs, including eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) via desaturase-mediated desaturation and elongation. Linoleic acid and ALA compete for the same enzyme systems for biosynthesis to long-chain PUFA (LCPUFA) and, importantly, for incorporation into membranes. Nonetheless, the conversation of ALA to EPA & DHA is relatively inefficient in humans, partly due to competition with n-6 linoleic acid for the rate-limiting enzyme for conversion into arachidonic acid [12]. Alternatively, EPA and DHA can be obtained directly from the diet by consuming oily fish such as salmon, tuna and mackerel.

LCPUFAs are precursors for eicosanoids which act as local and systemic mediators for coagulation, immune, allergic and inflammatory responses as well as having effects on BP and vascular reactivity. EPA is metabolized by the cyclooxygenase pathway into 3-series eicosanoids (prostaglandins, thromboxanes) and by 5-lipoxygenase into 5-series leukotrienes which have antagonistic physiological effects to the 2-series eicosanoids derived from arachidonic acid. DHA also gives rise to anti-inflammatory lipid mediators [13]. Leukotrienes and lipoxins derived from EPA and arachidonic acid are additional lipid mediators that modulate inflammation and serve as endogenous regulators of vascular tone and BP. Importantly, those derived from arachidonic acid are pro-inflammatory and pro-aggregatory agonists while those derived from EPA have opposing effects including anti-arrhythmic, hypolipidemic and hypotensive effects [14].

The beneficial effects of n-3 PUFAs have been explained by some authors in terms of a balance between total n-6 and n-3 FAs, rather than the absolute amount of each [15]. A high n-6/n-3 ratio has been hypothesized as being detrimental for human health, while conversely a ratio of ~1, estimated to be that consumed by our paleolithic forebears, is considered cardioprotective. During the last 150 years, a dramatic increase in the Western diet towards consumption of n-6 PUFAs paralleled with a decrease of n-3 PUFAs intake has resulted in a drastic shift resulting in average n-6/n-3 ratio between 15:1 and 20:1 [15].

Thus the dietary imbalance of LCPUFAs in favour of n-6 FAs can drive vascular and inflammatory responses, with consequent elevations of BP and other chronic diseases. Other vascular actions of n-3 PUFA are relatively poorly understood, but include changes in membrane structures, gene expression, and direct interactions with ion channels.

Anti-hypertensive Actions of n-3 PUFA

Studies have shown a number of antihypertensive effects of n-3 PUFA (Fig. 21.1). Consumption of n-3 PUFA reduces systolic and diastolic BP as well as resting heart rate in adults [16]. The reduced heart rate is thought to be due to direct effects on cardiac electrophysiology as well as indirect

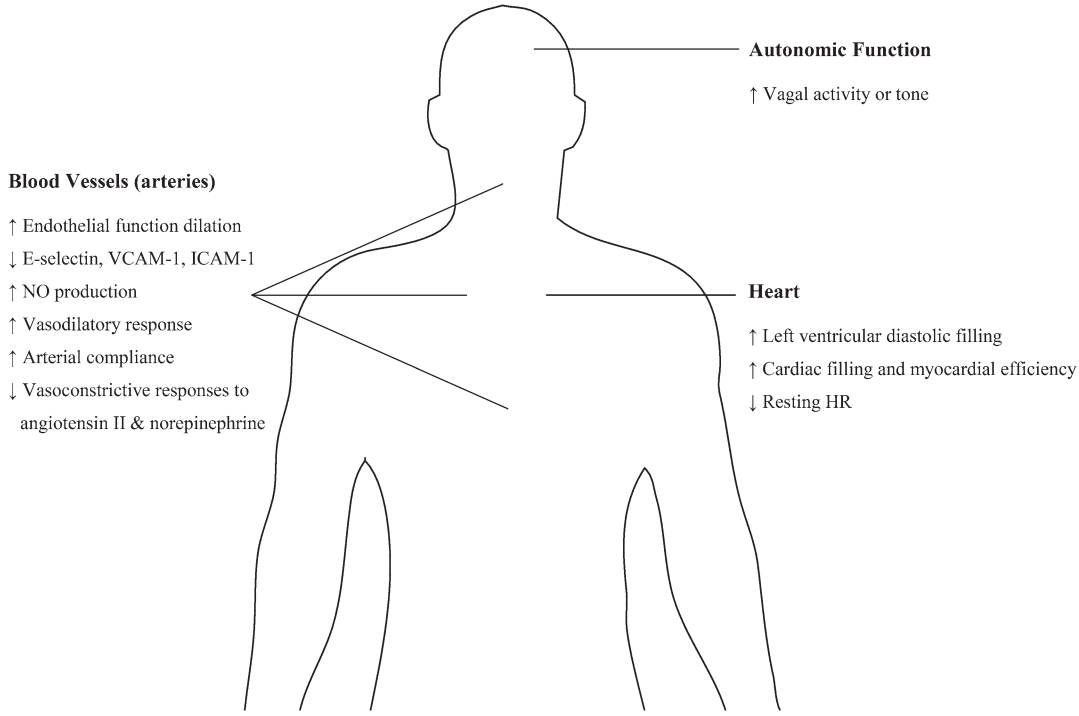

Fig. 21.1 Antihypertensive effects of omega-3 fatty acids: mechanistic evidence. n-3 polyunsaturated fatty acids have a number of antihypertensive effects, including improved arterial health, increased vagal activity and improved cardiac health. *ICAM-1* intercellular adhesion molecule-1, *NO* nitric oxide, *HR* heart rate, *VCAM-1* vascular cellular adhesion molecule-1

pathways [16], which include augmenting vagal tone and improving left ventricular diastolic filing [17]. Short-term trials indicate a number of vascular mechanisms of n-3 PUFAs including increased nitric oxide production, enhanced vasodilatory responses, attenuation of vasoconstrictive responses to angiotensin II and norepinephrine, improved arterial compliance and flow-mediated dilatation, a non-invasive marker of endothelial function [16]. N-3 LCPUFAs have beneficial effects on other aspects of endothelial health, including evidence that DHA decreases the expression of adhesion molecules on endothelial cells and monocytes, including E-selectin, intercellular cell-adhesion molecule-1 and vascular cell adhesion molecule-1. The magnitude of this effect is directly associated with the degree of incorporation of DHA into cellular phospholipids [18]. In human umbilical vein endothelial cells exposed to oxidized-LDL, EPA improves the balance between nitric oxide and reactive oxygen species [19]. Incubation with EPA also attenuates saturated fatty acid-induced generation of reactive oxygen species, expression of adhesion molecules and cytokines, activation of apoptosis-related proteins, and apoptosis in endothelial cells [20].

More recently, the cytochrome P450 enzymes have been proposed as targets of n-3 PUFAs to modulate vascular tone. It has become evident from several in vitro studies that n-3 PUFAs may exert potent vasodilatory effects as fatty epoxides metabolized by cytochrome P450 epoxygenase in the endothelium [21]. In isolated coronary arterial cells, DHA-derived epoxides were shown to activate calcium-activated potassium currents producing vasodilatation. Furthermore, the DHA-mediated dilatory effects were dose-dependent, increasing calcium-activated potassium currents by 5%, 170%, and 220% at 0.1, 0.3, and 1.0 mM DHA, respectively [22]. In recent experiments, n-3 PUFA-derived mono-epoxides was also reported to possess nearly 1000-fold greater potency than their precursors EPA or DHA in reducing effects of calcium overload in neonatal rat cardiomyocytes [23].

Observational studies and small trials suggest n-3 PUFA may improve autonomic function via augmentation of vagal activity or tone, although further studies are required to determine the appropriate dose required as either supplementation or as a component of dietary intake [16].

Taken together, these vascular and central mechanisms likely underpin the blood pressure lowering actions of n-3 PUFA.

Maternal n-3 PUFA and Offspring BP

The "fetal origins" hypothesis proposes that alterations in fetal nutrition result in developmental adaptations that permanently change structure, physiology, and metabolism, most likely influencing susceptibility to develop metabolic disorders, such as type 2 diabetes, cardiovascular disease, obesity and hypertension in later life [24]. It has been postulated that a deficiency in n-3 PUFAs in the perinatal period may be one such early life "insult" with hypertensive consequences.

Indeed, maternal DHA is readily transferred via the placenta, is an important component of breast milk, and is rapidly accumulated in the synapses during fetal development and early postnatal life. DHA comprise 30% of the phospholipid fatty acids within the cortex and 15% within the hypothalamus, a key brain centre that controls BP.

Maternal n-3 PUFA and Offspring BP: Experimental Animal Studies

To date, there is limited evidence from animal studies regarding the relationship between maternal n-3 PUFA and offspring BP. Armitage and colleagues [25] investigated the effects of maternal n-3 PUFA deficiency, in addition to the effects of post-weaning repletion of n-3 PUFA in the offspring, with subsequent offspring BP. Pregnant dams were given a diet either deficient in n-3 PUFA or supplemented with n-3 PUFA, throughout pregnancy and until weaning. At 9 weeks of age, half the supplemented offspring were crossed to the deficient diet and half the deficient offspring were subsequently supplemented, while the remainder continued to consume the diet to which their mother was originally allocated. In the adult offspring, mean arterial pressure (MAP) was highest in those fed the n-3 PUFA deficient diet throughout development and during post-weaning life (Fig. 21.2). Interestingly, the two groups with the lowest BP were those who were supplemented with n-3 PUFA throughout and those who were supplemented during pregnancy, but not post-weaning. This suggests that prenatal and early infancy is a critical period during which n-3 PUFA may modify adult BP in the rat, putatively via accumulation of n-3 PUFA in the developing nervous system.

A similar study investigating n-3 and n-6 PUFA, and their ratios, found that BP was elevated in the offspring of those dams fed either a diet enriched in n-3 or n-6 PUFA prenatally [26]. Only male offspring from dams given the diet containing both n-6 and n-3 PUFA had elevated blood pressure, suggesting that prenatal fatty acid balance may be important in influencing BP in a gender-specific manner.

Observations from Armitage and colleagues offer some clues as to mechanisms by which PUFA and its composition affect early development and BP control. Rats exposed to n-3 PUFA deficiency while in utero and during infancy were found to over ingest sodium and consequently consume lower amounts of water, as part of a sodium and water deprivation challenge [27]. Such behaviour is consistent with abnormalities in sodium and osmoreceptors as well as the renin angiotensin mechanism, both of which influence BP and hydromineral balance. N-3 PUFA deficiency is also found to affect phototransduction in the retina, which share similar receptor morphology as the angiotensin II receptor [25]. These changes may have emerged due to developmental changes in the membrane bound receptors.

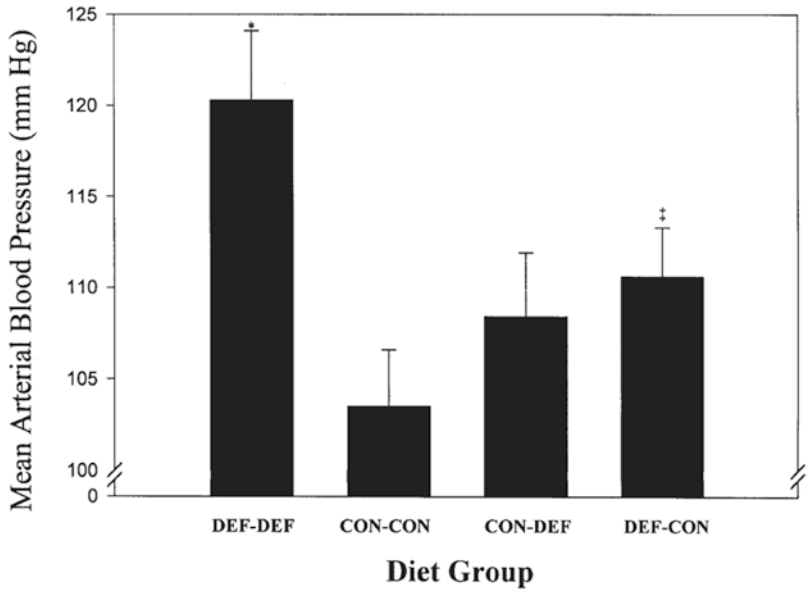

Fig. 21.2 Effect of dietary fatty acid supply on mean arterial blood pressure (MAP). Cross over diet was implemented at 9 weeks of age, where half the supplemented (CON) offspring were crossed to the deficient diet (CON-DEF) and half the DEF offspring were crossed to the control diet (DEF-CON). The remaining rats continued on their initial diets (CON-CON and DEF-DEF). This study demonstrated that (1) the highest MAP occurred in animals raised and maintained on a diet deficient in n-3 PUFA (DEF-DEF); and that (2) early n-3 PUFA deficiency with subsequent n-3 PUFA supplementation at 9 weeks of age (DEF-CON) still resulted in raised blood pressure when compared to those who received PUFA supplementation throughout (CON-CON). This is consistent with a deficiency in n-3 PUFA during the prenatal and perinatal period affecting adult hemodynamics. *Significantly higher than all other groups ($P < 0.05$); ‡ significantly higher than CON-CON ($P < 0.05$). n-3 PUFA, omega-3 polyunsaturated fatty acids (Reprinted from Armitage et al. [25] Copyright © 2003 by AOCS Press, with permission of Springer)

Maternal n-3 PUFA During Pregnancy and Offspring BP: Human Studies

All of the n-3 and n-6 PUFAs accumulated by the fetus must ultimately be derived from the mother by placental transfer and studies have demonstrated the preferential selectivity in the placental membrane binding sites for LCPUFAs, in particular DHA [28].

Despite the functions of n-3 PUFAs in fetal and newborn neurodevelopment and inflammation, there is only a relatively small body of evidence describing the associations of maternal n-3 PUFA intake with BP or measures of cardiovascular health in the offspring (Table 21.1).

In the Generation R Study, an observational cohort of 4455 mothers and children [29], higher maternal n-3 PUFA and lower n-6 PUFA concentration in the second trimester of pregnancy was associated with lower SBP but not with DBP in the offspring during childhood. In models adjusted for gestational age at sampling, pregnancy and childhood factors, and sex and age of the child, both higher total maternal n-3 PUFA concentration and DHA concentration were found to be associated with lower SBP in the children at 6 years of age (differences: −0.28 [95% CI −0.54, −0.03] and −0.29 mmHg [95% CI −0.54, −0.03] per SD increase of n-3 PUFAs [one SD is 1.5 wt%] and DHA [one SD is 1.1 wt%] respectively) [29]. Conversely, a higher maternal n-6 PUFA was associated with a higher childhood SBP (difference: 0.36 mmHg [95% CI 0.09, 0.62] per SD increase of total n-6 PUFA [2.5 wt%]). Furthermore, a higher n-6/n-3 ratio was associated with an increased childhood SBP, but not with DBP. In separate analyses of the Generation R cohort, a higher maternal n-6/n-3 PUFA ratio was associated with a higher childhood total body fat mass percentage, android:gynoid fat

Table 21.1 Outcomes of relevant studies examining the association between maternal n-3 PUFA intake and offspring BP

Ref no.	Study design	Subject details	Diet/intervention	Key outcomes
[33]	Randomised controlled trial	n = 46 pregnant women	Maternal DHA supplementation (600 mg/day; n = 22) or placebo (n = 24) from 14 weeks GA until birth	Weak evidence for lower fetal heart rate at 24, 32, and 36 weeks GA in mother supplemented with DHA compared to placebo (P = 0.095)
[34]	Randomised controlled trial	n = 180 mother-child pairs	Maternal supplementation with fish oil containing 2.7 g/day fish oil (n = 108) or olive oil (n = 72) or no supplementation (n = 214) from 30 weeks GA until birth	Maternal supplementation with fish oil during the last trimester of pregnancy was not associated with differences in BP, hear rate or heart rate variability in the offspring at 19 years of age, when compared to those assigned to olive oil [differences: 2 mmHg (95% CI: −1, 4) for SBP and 1 beat per minute (95% CI: −2, 4) for HR]
[29]	Cohort study (Generation R population-cohort study)	n = 4455 mother-child pairs	Maternal second trimester plasma concentrations of n-3 and n-6 PUFA (wt% total fatty acids)	Higher plasma concentrations of maternal n-3 PUFA and DHA was associated with lower SBP in the offspring at 6 years of age [differences: −0.28 mmHg (95% CI: −0.54, −0.03) and −0.29 mmHg (95% CI: −0.54, −0.03) per SD higher n-3 PUFAs and DHA respectively]
				Higher maternal n-6 PUFA was associated a higher childhood SBP [difference: 0.36 mmHg (95% CI: 0.09, 0.62) per SD higher n-6 PUFA] at 6 years of age
[31]	Cohort study (Avon Longitudinal Study of Parents and Children; ALSPAC)	n = 6944 mother-child pairs	Data from maternal Food Frequency Questionnaires based on the diet in pregnancy	In minimally adjusted models, maternal n-3PUFA intake based on the current diet in the last trimester of pregnancy was inversely associated with SBP in the children at 7.5 years of age (P = 0.04), effects were lost after adjusting for current anthropometry, maternal and social factors, birth weight and gestation (P = 0.7)
[32]	Cohort study (Southampton Women's Survey)	n = 234 mother-child pairs	Data from maternal Food Frequency Questionnaires based on diet in pregnancy	Higher oily fish consumption in late pregnancy was associated with reduced aortic pulse wave velocity in the offspring at 9 years of age (−0.084 m/s per portion per week; 95% CI, −0.137, −0.031)

(continued)

Table 21.1 (continued)

Ref no.	Study design	Subject details	Diet/intervention	Key outcomes
[35]	Cohort study	n = 443 mother-child pairs	Data from maternal Food Frequency Questionnaires based on diet in pregnancy	Maternal n-3 PUFA intakes expressed as quintiles in the second trimester of pregnancy was not associated with BP, heart rate, or heart rate variability in the offspring at 20 years of age
[45]	Randomised controlled trial	n = 98 mother-child pairs	Lactating mothers randomised to 4.5 g/day fish oil (n = 39) or olive oil (n = 30), or a non-randomized group consuming a high fish diet (n = 29) during the first 4 months after delivery	Blood pressure, pulse wave velocity, or heart rate variability did not differ between infants of mothers supplemented with fish oil or olive oil at 2.5 years of age
[46]	Randomised controlled trial	n = 147 newborn infants	Newborn infants randomised to a formula containing LCPUFA supplementation (n = 71) or nutritionally similar formula without LCPUFA (n = 76) for 4 months and followed up at 6 years	Mean BP was – 3.0 mmHg lower ([95%CI −5.4, −0.5]; P = 0.02) and DBP was −3.6 mmHg lower [95%CI −6.5, −0.6]; P = 0.02) than the non-supplemented group at 6 years of age
[47]	Randomised controlled trial	n = 83 term 9 month old infants	Infants randomised to 5 mL fish oil (n = 39) or no fish oil (n = 44) daily for 3 months	SBP was lower in the fish oil supplemented group at 12 months compared to those infants in the control group [mean difference−6.3 mmHg (95% CI, −0.9, 11.7 mmHg)]
[44]	Self-selected intervention study	n = 102 mother-child pairs	Breast milk (n = 31) vs. milk-based formula (n = 39) vs. soy-based formula without DHA (n = 12) vs. soy-based formula + DHA (n = 30) from birth to 6 months	Increased heart rate and decreased heart rate variability measures were observed in infants fed the DHA-deficient diet compared to the other diets

BP blood pressure, *DHA* docosahexaenoic acid, *GA* gestational age, *LCPUFA* long chain polyunsaturated fatty acids, *n-3 PUFA* omega-3 polyunsaturated fatty acids, *n-6* omega-6 polyunsaturated fatty acids, *SBP* systolic blood pressure

mass ratio, and abdominal preperitoneal fat mass area adiposity at 3, 4 and 6 years of age [30], potentially driving the observed association with BP.

The Avon Longitudinal Study of Parents and Children reported an inverse association between maternal n-3 PUFA intake assessed during the third trimester of pregnancy with offspring SBP at 7.5 years of age in 6944 mother-child pairs ($P = 0.04$), although this effect was lost after adjusting for current anthropometry, maternal and social factors, birth weight and gestation ($P = 0.7$) [31]. There were no significant associations between maternal dietary n-3 PUFA intake and offspring DBP.

More recently, the Southampton Women's Survey reported that higher oily fish consumption in late pregnancy was associated with reduced aortic stiffness, assessed by pulse wave velocity, in the child at 9 years of age (−0.084 m/s per portion of oily fish consumed per week; 95% CI −0.137, −0.031) independent of the child's current oily fish consumption [32]; however there was no association with offspring BP or heart rate.

Promising findings on DHA supplementation and fetal heart rate have been reported. A randomised controlled trial involving maternal supplementation of 600 mg/day of DHA commenced at 14 weeks gestation was found to lower heart rate at 24, 32 and 36 weeks gestational age compared to placebo ($P = 0.095$) [33]. Furthermore, there was evidence that DHA intake during the last two trimesters of pregnancy results in more responsive autonomic function in the offspring.

Follow-up studies examining effects in adolescence have also yielded discrepant findings. A randomised controlled trial in which 180 pregnant women were supplemented with marine n-3 LCPUFAs in the last trimester of pregnancy showed no association with BP, heart rate or heart rate variability, a marker of autonomic function, in the offspring at 19 years or age [34]. Similarly, marine-derived dietary n-3 LCPUFA expressed as quintiles of energy-adjusted intake during the second trimester of pregnancy showed no association with SBP, DBP, heart rate or heart rate variability in 443 offspring at 20 years of age in a Danish cohort [35].

Maternal n-3 PUFA During Lactation and Offspring BP: Human Studies

For the infant, PUFAs are essential during the perinatal period where there is rapid growth and development of new tissues and organ systems, and is primarily sourced from breast milk in nursing infants. The lactating mammary tissue synthesises FAs intracellularly from a supply of substrates extracted from the maternal plasma. The lipid drops formed in the mammary epithelial cells are secreted into the milk by exocytosis or association with the plasma membrane bilayer [36]. While the level of n-6 arachidonic acid is relatively constant in human milk, the EPA and DHA levels are variable and dependent on the maternal nutritional habits [37], with maternal intake of fish oil, DHA, or DHA-enriched foods effectively increasing both maternal and neonatal n-3 PUFA status [38].

Studies linking breast milk intake in infancy to lower BP during childhood have been reported. Breastfeeding has also been associated with consistent reduction in obesity risk later in childhood [39], with likely benefits for BP, although the n-3 PUFA content of breast milk is likely not a major factor contributing to this lower risk of offspring adiposity. LCPUFAs are present in breast milk but were not routinely available in infant formula until the early 2000s. As infants have limited ability to synthesise DHA, infants fed unsupplemented formula may experience a relative deficiency of LCPUFAs compared to breast-fed infants [40].

Longitudinal observational studies in term infants have demonstrated that children who were breast-fed for at least 3 months have lower SBP and DBP during later childhood and adolescence compared to children who were formula fed [41, 42]. Fifteen year old children born preterm and randomised during infancy to being fed banked breast milk, had lower SBP and DBP compared to those who were randomised to infant formula [43].

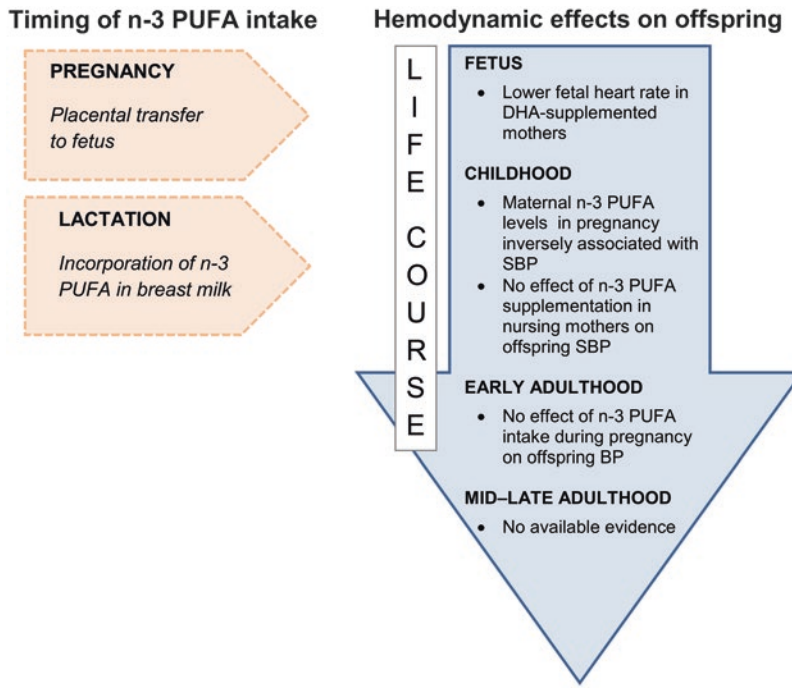

Fig. 21.3 Summary of evidence of maternal n-3 PUFA intake and offspring BP across the life course. Current evidence linking maternal intake of n-3 PUFA and lower offspring blood pressure is inconclusive. While some observational studies have shown inverse associations, others have not, and intervention studies have reported no effect. There is a lack of evidence from prospective randomised trials of maternal n-3 PUFA with offspring blood pressure as a prespecified outcome. *BP* blood pressure, *DHA* docosahexaenoic acid, *n-3 PUFA* omega-3 polyunsaturated fatty acids, *SBP* systolic blood pressure

Increased heart rate and decreased heart rate variability were found in infants fed a DHA-deficient milk formula compared to those fed DHA-supplemented formula or breast-fed infants [44]. In contrast, maternal supplementation with marine n-3 PUFAs during the first 4 months of lactation had no effect on BP, arterial stiffness or heart rate variability of the offspring at 2.5 years of age [45].

Postpartum intervention in infancy has proven effective in some randomised trials. A study of 71 newborn infants who were randomised to a formula with LCPUFAs reported a significantly lower DBP at 6 years of age compared to those children who were fed a nutritionally similar formula in infancy but devoid of LCPUFAs (mean difference −3.0 mmHg [95% CI −5.4, −0.5 mmHg]) [46]. The association was weaker for SBP (mean difference −2.3 mmHg [95% CI −5.3, 0.7 mmHg])]. In late infancy supplementation with marine n-3 LCPUFA (924 mg/day) in 9 month old infants for 3 months resulted in a lower SBP compared to those infants that did not receive n-3 PUFA (mean difference −6.3 mmHg [95% CI −11.7, −0.9,mmHg]). No effects were observed for DBP or MAP [47]. Although these do not directly study the effect of maternal n-3 PUFA intake, these trials of formula supplemented with n-3 PUFA compared with deficient formula, can be used as proof-of-concept of the potential haemodynamic effects of n-3 PUFA intake during infancy per se. It is not unreasonable to posit that similar findings could result in breast-fed infants of mothers consuming an appropriate amount of n-3 PUFA. Importantly, these infants would also obtain the other well-described health benefits of being breast-fed, including reduced risk of later obesity (Fig. 21.3).

While there are currently no widely recognised guidelines for intake of n-3 PUFA during pregnancy and lactation, there are well established recommendations for the general population of two servings of oily fish per week providing an average of ~100 to 250 mg per day n-3 PUFAs of which

50–100 mg is from DHA [48]. It is likely that there are increased n-3 PUFA requirements during pregnancy, however most pregnant women likely do not meet the increased demand in part due to competing recommendations to limit oily fish consumption to a maximum of two servings per week due to concerns of their mercury content. For women consuming a solely or predominantly plant-based diet, there are other concerns in meeting these requirements. While plant-based foods can be rich in ALA, only a small amount of these short-chain n-3 PUFA appear to be endogenously converted to LCPUFA, and these dietary patterns provide only small amounts of EPA and DHA [48]. Accordingly, the use of supplements may be warranted in some women during pregnancy and lactation, who are otherwise unable to obtain sufficient n-3 LCPUFA from dietary sources.

The ability of n-3 PUFA in the fetus or during early infancy to reduce later BP may also depend on the background risk of the individual, particularly relating to the presence of other early life risk factors for hypertension. A recent randomized trial of fish oil supplementation over the first 5 years of life in children who were not considered to be at risk of cardiovascular disease, found evidence of interaction by birth weight, such that those with the lowest birth weight had less severe subclinical atherosclerosis if they had been allocated to receive the fish oil supplement [49]. Similar findings have since been demonstrated for BP, with intake of both plant and marine-derived n-3 PUFA being associated with lower BP [50]. It is plausible that those born with impaired fetal growth in particular, are a group in which sustained n-3 PUFA intake postnatally may be beneficial, given that birth weight is inversely associated with later BP, that on average children born with impaired fetal growth are more likely to have been exposed to lower n-3 PUFA in utero, and that impaired fetal growth is associated with lower serum DHA and EPA levels later in life [50] (Fig. 21.4). Indeed, the above studies reporting

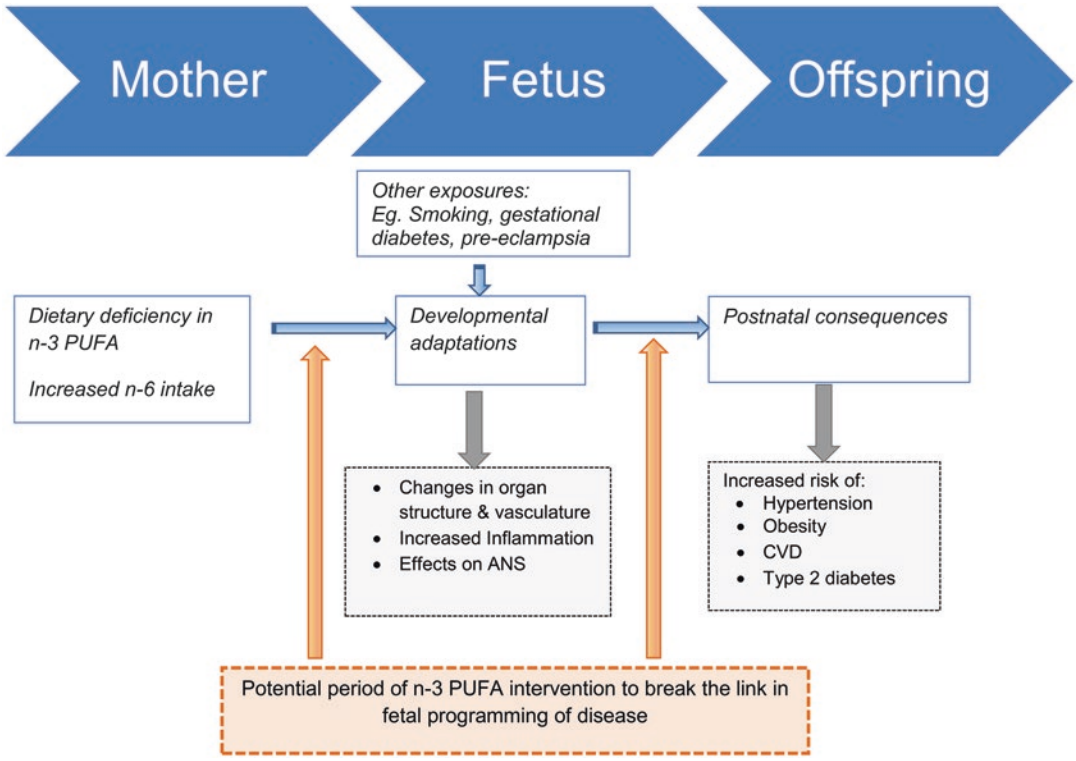

Fig. 21.4 Schematic proposed pathways linking n-3 deficiency with hypertensive disorders: potential timing for n-3 PUFA interventions. Theoretical time points, including while in utero and postnatal, during which n-3 PUFA interventions could be applied to reduce or prevent the chronic disease outcomes of fetal programming. *ANS* autonomic nervous system, *CVD* cardiovascular disease, *n-3 PUFA* omega-3 polyunsaturated fatty acids, *n-6* omega-6 polyunsaturated fatty acids

the association of n-3 PUFA intake and BP lowering properties in those born with impaired fetal growth, found that similar associations were either not present or markedly weaker in those born with healthy birth weight [50].

Conclusions

Accordingly, while a link between maternal intake of n-3 PUFAs and lower offspring BP is mechanistically plausible, the evidence thus far is mixed and inconclusive, and limited principally by the lack of prospective randomised trials with BP as a pre-specified outcome.

There are a number of issues that will need to be duly considered in the design of such trials, and any recommendations that flow therefrom. What is the best vehicle for n-3 PUFA interventions in pregnant women? Consuming oily fish is generally recommended outside of pregnancy, for cardiovascular disease prevention, predominantly due to the inherent substitution of such a meal for a likely less healthsome meal. However, mercury levels in consumed fish are an important consideration, particularly during pregnancy, and as such consumption of species including swordfish and shark should be limited.

Fish oil supplements may be more amenable to some women, due to food preferences and tolerances, and most of the widely available supplements are low-mercury or mercury-free. Algae-derived DHA preparations overcome these concerns, and are also a suitable source of n-3 LCPUFA for women adhering to a plant-based diet. People adhering to such diets have higher ALA intake on average, the role of which during pregnancy remains poorly described.

Finally, identifying groups of women whose offspring will have the greatest likelihood of achieving clinically meaningful reductions in BP resulting from n-3 PUFA intake during pregnancy is an on-going challenge. As noted, such offspring are likely to be those at the highest risk of elevated BP due to an adverse maternal-fetal environment, including but not limited to those born premature or with fetal growth restriction, although clinical identification of women at risk of these outcomes early enough in pregnancy to enable the intervention to have a meaningful effect, remains challenging.

Yet despite these challenges, given the global burden of hypertensive-disorders and the strong evidence for developmental origins of hypertension, maternal n-3 intake is a potentially powerful and meaningful strategy to normalize offspring BP which warrants further careful and robust evaluation.

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