BLOOD PRESSURES IN PREGNANCY: DETERMINANTS AND CONSEQUENCES

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DECLARATION

I hereby declare that the thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

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Lim Wai Yee

12 May 2015

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Summary

Maternal blood pressures are important arterial marker for hypertension and cardiovascular outcomes in the women and their offspring. While the determinants and consequences of hypertension in pregnancy are well studied in the literature, few have examined the components and the quantitative nature of blood pressures during pregnancy, particularly in Asian women. Therefore, the aims of this thesis are twofold: (1) to examine the influence of maternal lifestyle factors (adiposity and plasma polyunsaturated fatty acids [PUFAs]) on blood pressures and (2) the impact of blood pressures on offspring health outcomes (size at birth and early childhood blood pressures) in a cohort of Southeast-Asian Chinese, Malay and Indian women.

Data were obtained from 1162 pregnant women who were recruited for the "Growing Up in Singapore Towards healthy Outcomes" (GUSTO). Maternal blood pressures (peripheral and central) were measured using automated oscillometric device and radial pulse wave analysis during GUSTO study follow-up at 26 - 28 weeks gestation. Anthropometric measures and blood samples were also taken at the same visit. Of the 1162 GUSTO study participants, 829 (71.3%) had evaluable peripheral and central blood pressures, and they were follow-up till delivery and at 36 months post-delivery. These women were included in the analysis of the four studies included in this thesis.

In determining the influence of maternal lifestyle factors on blood pressures, cross-sectional analysis of GUSTO data on the respective influences of maternal adiposity and plasma PUFA levels on peripheral and central blood

pressures measured at 26 - 28 weeks gestation were performed. Findings showed positive maternal adiposity-blood pressure relations and inverse relations between maternal n-3 PUFA and blood pressures. An ethnic modification effect was also observed for the two relations, with stronger estimates observed in Chinese women than Malay or Indian women.

On the consequences of higher maternal blood pressures on health outcomes (size at birth and early childhood blood pressures) in the offspring, analysis of data on women with complete information at delivery and 36 months post-delivery were performed. First, on the outcome of offspring size at birth, findings showed that higher maternal peripheral and central blood pressures were associated with smaller offspring and an increased risk of delivering offspring of low birth weight and small for gestational age. Maternal adiposity modified the relations, with stronger inverse association in normal weight women than overweight or obese women. Although the effect modification by maternal ethnicity was not statistically significant, Chinese women with higher blood pressures tended to have smaller offspring. Second, on the outcome of early childhood blood pressures, findings showed positive maternal-offspring blood pressure relations which persisted even in normotensive women who were free of hypertension during pregnancy. No significant effect modification by maternal ethnicity was observed.

These findings suggest that maintaining blood pressure compliance during pregnancy is important to ensure optimal fetal growth and development. Therefore, preventive strategies incorporating healthy lifestyle during pregnancy, such as maintaining appropriate range of weight gain and healthy

diet with optimal n-3 fatty acids intake may be useful adjuncts to current antenatal management of pregnant women.

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
ALA	α-linolenic acid
ALSPAC	Avon Longitudinal Study of Parents and Children
BMI	Body mass index
CI	Confidence interval
DBP	Diastolic blood pressure
DM	Diabetes mellitus
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid;
GDM	Gestational diabetes
GH	Gestational hypertension
GUSTO	**G**rowing **U**p in **S**ingapore **T**owards healthy **O**utcomes
HUNT Study	**H**else**u**ndersøkelsen i **N**ord-**T**røndelag) Study
IOM	Institute of Medicine
LBW	Low birth weight
LA	Linoleic acid
MAP	Mean arterial pressure

MUFA	Monounsaturated fatty acids
OR	Odds ratio
PC	Phosphatidylcholine
PE	Preeclampsia
PP	Pulse pressure
PUFA	Polyunsaturated fatty acids
RCT	Randomised clinical trials
RR	Risk ratio
SBP	Systolic blood pressure
SD	Standard deviation
SFA	Saturated fatty acid
SGA	Small for gestational age
WHO	World Health Organization

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CHAPTER 1

BACKGROUND

1.1 Introduction

Hypertension is a global public health issue, contributing to the burden of heart disease, stroke, kidney failure, premature disability and mortality among women,[1-4] According to World Health Organization report in 2013, hypertension accounts for 45% of deaths from heart disease and 51% of deaths due to stroke.[5] In women, hypertension during pregnancy has been shown to have higher risk to cardiovascular and metabolic outcomes later on in life.[1, 2] Metaanalysis from a systematic review of more than 43 studies has demonstrated that women with preeclampsia have a 2.3 fold increase risk to a clinical diagnosis of cardiovascular disease or a fatal outcome; and a 1.8 fold to hypertension and 2 fold to diabetes in later life.[2]

In pregnancy, cardiovascular adaptation is one of the major physiological changes, occurring as early as 8 weeks gestation.[6] In the pregnant women, reduction in blood pressures from systemic vasodilation and reduced vascular resistance are important physiological changes as it maintains the circulatory system during pregnancy,[6, 7] while ensuring adequate fetal supply for optimal growth and development.[8-10]

By convention, blood pressures from the brachial arm is often taken, as central blood pressures are not amenable to non-invasive measurement. But with the advent of non-invasive acquisition of pulse wave analysis, central blood pressures may be estimated with high reliability[11-13] and reproducibility.[14-16] In the literature, central systolic and pulse pressures, as indicators of cardiovascular adaptation, have been suggested to be surrogate markers of arterial stiffness.[17] There are pressure differences between central

and peripheral sites, for example, between the aorta and brachial artery, and this is due to amplification effects which is influenced by aging and medication.[18]

Blood pressure follows a normal distribution in the population and have a graded relationship to cardiovascular outcomes.[19] Findings from epidemiological studies suggest that maternal blood pressures during pregnancy are associated with systemic disorders, genetic and environmental factors.[20, 21] In turn, higher maternal blood pressures are also associated with adverse health outcomes in the women and their offspring.[20, 22] However, findings from these studies are based on peripheral blood pressures, and few have evaluated central blood pressures. Moreover, few have examined the continuous distribution of maternal peripheral and central blood pressures. Study findings based on prevailing definitions of hypertension which depend on peripheral blood pressures thresholds for treatment benefits, may overlook the properties of systolic, diastolic or pulse pressures, and are of limited value for epidemiologic purposes.

Given the public health importance of hypertension, targeting women early, before or during pregnancy through lifestyle modification measures may be a useful strategy in reducing maternal risk to hypertension during pregnancy and later on in life. Therefore, the focus of this thesis includes the influence of lifestyle factors on maternal adiposity and nutrition (plasma polyunsaturated fatty acids) as determinants of maternal blood pressures; and in turn, the consequences of maternal blood pressures on offspring size at birth and early childhood blood pressures.

1.2 The Arterial System in Pregnancy

The arterial tree in the circulatory system is thought to be a static, elastic system, and the pressure along the arterial tree is determined by the physical factors, being the arterial blood volume and arterial compliance.[23] These physical factors, in turn, are affected by the physiological factors which include cardiac output (as determined by heart rate and stroke volume) and peripheral resistance.[23] During pregnancy, the cardiac chambers dilates,[6, 24] and the arterial tree undergoes structural changes rendering it to be more compliant.[25] The structural changes include fragmentation of the reticular fibers, a reduction in acid mucopolysaccharides and normal corrugation of elastic fibers, hypertrophy and hyperplasia of smooth muscle cells,[26] and increase in aortic diameter.[27] Collectively, these changes enhance the arterial distensibility and capacitance,[23, 28] thereby increasing arterial compliance.[7]

1.2.1 Physiological Changes and Arterial Blood Pressures

Cardiovascular changes in pregnancy are characterized by increased blood volume and cardiac output.[6] Concurrent decrease in systemic vascular resistance and blood pressures occur in proportion to these increases.[6, 20] Overall, these shifts reflect the enhanced aortic distensibility modulated by the endothelium-dependent factors such as nitric oxide synthesis upregulated by oestradiaol and prostaglandins.[6, 20, 24] It is well accepted that peripheral blood pressures start to decrease in early pregnancy till mid-trimester at 22-24 weeks gestation and gradually towards pre-pregnancy level until term.[6, 29, 30]

Systolic and diastolic blood pressures reflect the peak and trough of the blood pressure at each cardiac cycle (Figure 1-1).[31, 32] Pulse pressure,

measured as the difference between systolic and diastolic blood pressures, is dependent on the arterial blood volume and arterial capacitance. It is therefore function of arterial compliance.[23]

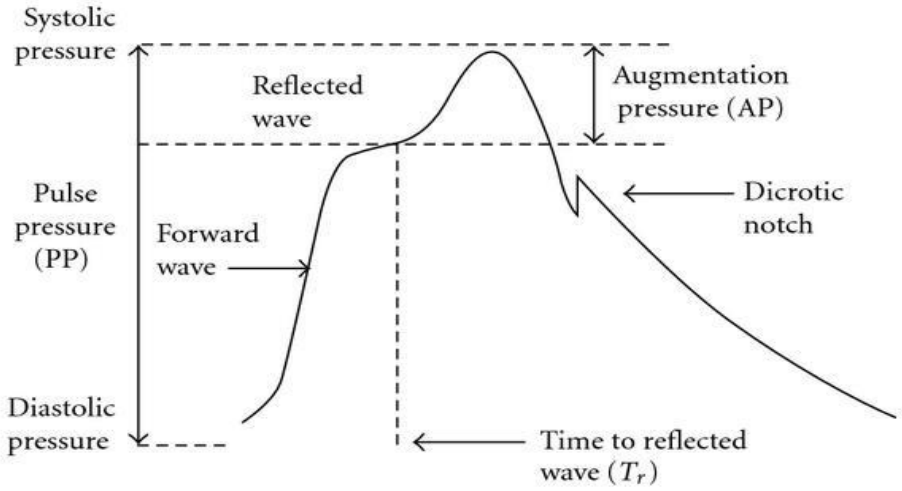

Figure 1-1. Aortic Pressure Waveform within a Cardiac Cycle

Source[32]: Van Varik B, Rennenberg R, Reutelingsperger C, Kroon A, de Leeuw P, Schurgers LJ. Mechanisms of arterial remodeling: Lessons from genetic diseases. *Frontiers in Genetics*. 2012;3

Due to pulse wave amplification effects, systolic and pulse pressures differ along the arterial tree,[28, 33, 34] and therefore, they reflect the pulsatile components of blood pressure.[35] Conversely, diastolic blood pressure reflects the tonic component of blood pressure as it is unaffected by amplification effects.[36] Unlike systolic or pulse pressures, diastolic blood pressure is assumed to be constant along the arterial tree.

During a cardiac cycle, pressure waves are amplified as they travel from the aorta to the periphery, and thereby augmenting the systolic and pulse pressures in the periphery.[23, 37] Pressure wave amplification is more marked in healthy conduit arteries, particularly in the young, at a brachial to aortic pulse pressure ratio of 1 to 5.[35] With aging or arterial diseases, pressure wave

amplification decreases and the gap between peripheral and central pressures narrows.[23, 35]

Although physiological studies have found that blood pressures change continuously within a cardiac cycle, peripheral systolic and diastolic blood pressures taken from the brachial artery is thought to reflect the pressure throughout the large conduit arteries.[30, 38-41] During pregnancy, changes central blood pressures occur in parallel with peripheral brachial blood pressures across pregnancy trimesters.[29, 30] However, compared to peripheral brachial pressures, pregnancy associated changes in central aortic pressure are more marked due to pulse wave amplification effects.[30, 41]

1.2.2 Basic Mechanisms in Arterial Hypertension

Hypertension is essentially due to increased peripheral resistance and decrease in arterial distensibility.[20, 23, 28] At higher blood pressures or hypertension, the central arteries are larger and stiffer, thereby leading to higher aortic impedance.[28] The higher amplitude of the incident pressure wave at ventricular ejection and earlier return of the reflected pressure wave from the peripheral arteries contribute to a disproportionate increase in systolic blood pressure and pulse pressure (Figure 1-2).[23, 28, 42, 43]

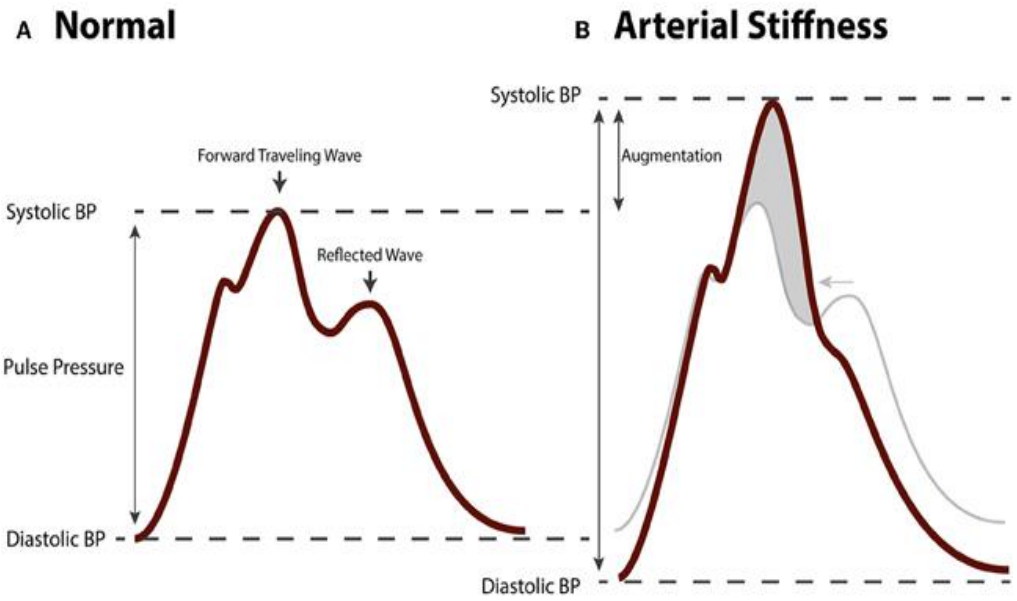

Figure 1-2. Aortic Pressure Waveforms in Normotensive Person (A) Person with Arterial Stiffness (B).

Source[43]: Stoner L, Young JM, Fryer S. Assessments of arterial stiffness and endothelial function using pulse wave analysis. *Int J Vasc Med*. 2012;2012:903107

In persistent hypertension, arterial degeneration occurs prematurely and accelerates as seen in aging.[28] The central aorta becomes larger and stiffer to a greater extent, while the peripheral arteries undergoes intimal hyperplasia, atherosclerosis and impaired endothelial function.[20, 28] Reactive vascular changes in the kidneys may also occur, leading to further vasoconstriction, salt retention and increase blood volume through the activation of the renal-angiotensin aldosterone system.[21, 28, 32]

In pregnancy, the key features underlying the development of preeclampsia, a severe form of pregnancy hypertension, are inadequate placentation, exaggerated inflammatory response and endothelial dysfunction.[21, 44] To date, the pathophysiology underlying preeclampsia is unknown and various propositions from inadequate placentation, hypoxia, immune maladaptation, renin-angiotensin-aldosterone imbalance, excessive

oxidative stress to genetic susceptibility have been proposed.[44, 45] As inadequate placentation is key to preeclampsia, there has been considerable focus on abnormal placentation and the development of preeclampsia.[6, 21, 45, 46]

At the initial stage of disease development, inadequate placentation is observed in the first half of the pregnancy and thereafter, uteroplacental ischaemic changes is observed, causing abnormal placental perfusion. In turn, the ischaemic placenta leads to increase in cytokines release or deportation of syncytiotrophoblast particles into the maternal circulation.[6, 46] These factors triggers a widespread microvascular damage and endothelium dysfunction in the pregnant women and consequently the overt presentation of hypertension and proteinuria.[6, 45]

1.2.3 Classifications of Pregnancy Hypertension

Current definitions for pregnancy hypertension based on guidelines from the Australasian Society for the Study of Hypertension in Pregnancy (ASSHP),[47] National Institute for Health and Clinical Excellence (NICE)[48] and American's College of Obstetricians and Gynaecologist (ACOG),[49] is defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg; and it is differentiated by gestation, with hypertension occurring before 20 weeks gestation as chronic hypertension and those after as gestational hypertension (previously known as pregnancy induced hypertension). Preeclampsia, as defined by hypertension and proteinuria (≥ 0.3g protein in a 24-hour urine specimen) occurring after 20 weeks gestation, is a spectrum of disorder as it may be accompanied by other signs and symptoms involving the renal, liver, neurological, haematological and fetal growth restriction.[47-49]

Although these guidelines share similarities in the definition for gestational hypertension and preeclampsia, there are subtle differences. For example, the definition for preeclampsia by ASSHP[47] included hypertension and any criteria from proteinuria to any other clinical signs and symptoms involving the renal, liver, neurological, haematological and fetal growth restriction; but not the NICE[48] and ACOG[49] guidelines. Moreover, past criteria such as edema and sudden increase in systolic or diastolic blood pressures based on the 30-15 mmHg rule were no longer included in the definition of preeclampsia but have been retained in the guidelines as important signs of preeclampsia. More specifically, (1) sudden or rapid development of edema is abnormal and women should be aware of the need to seek medical attention in the NICE[48] and ASSHP[47] guidelines and (2) sudden change in blood pressures is retained in the ACOG[49] guideline only, with an emphasis on warranting further investigation.

1.2.4 Methods of Measuring Arterial Blood Pressures

By convention, blood pressures are taken from the non-dominant arm at the brachial artery using either a sphygmomanometer or an oscillometric device.[50] When blood pressures are determined using a sphygmomanometer, the systolic and diastolic blood pressures may be estimated by the palpatory or auscultatory method on the brachial artery based on the Korotkoff sounds.[31, 50] However, like all measuring devices, the sphygmomanometer requires calibration.[50, 51]

In contrast to the sphygmomanometer, the oscillometric device measures blood pressures by utilizing a cuff with sensing and occluding functions.[31, 50,

[51] It is also simpler and less operator dependent.[36] However, oscillometric devices are suitable only for persons with regular and stable pulse.[51] Depending on the size of the oscillation, blood pressures may be underestimated and in persons with very low blood pressures,[31, 50] blood pressures may not be recordable[51] For example, in elderly person with stiff arteries and wide pulse pressure, the mean arterial pressure will be underestimated.[50]

Technological advances have enabled the estimation of central blood pressures through a non-invasive approach by measuring the carotid or radial artery pulse wave forms.[18] From these wave forms, central blood pressures may be estimated through the general transfer functions, late systolic peak pressure or the n-point moving average technique.[31, 52, 53] However, the non-invasive measurement of central blood pressures is device dependent and requires calibration from peripheral blood pressures.[38, 54-58]

Current methods in measuring blood pressures are susceptible to measurement errors compared to the gold standard of invasive measure of intra-arterial blood pressures.[36, 50, 51, 59] In spite of the variation between brachial and intra-arterial blood pressures, brachial blood pressures continue to be an important marker for cardiovascular outcomes[28, 50, 60] as it has been widely used in research and is well established in clinical practice. On the other hand, non-invasive measures of central blood pressures have shown good agreement with invasive measure of intra-arterial blood pressures.[54] And over the last 15 years, non-invasive measures of central blood pressures are gaining widespread attention as there is evidence to suggest its role in prediction of cardiovascular outcomes.[61-64]

1.3 Literature on Arterial Blood Pressures during Pregnancy

Blood pressures during pregnancy are known to be determined by socio-demographic, lifestyle and systemic disorders.[20, 28, 65-67] Among these factors, maternal lifestyle factors such as adiposity, diet, smoking, alcohol consumption and physical activity have important public health implications as they are modifiable risk factors for hypertension.[28, 68] Amongst these lifestyle factors, maternal adiposity and diet (being the intake of fatty fish or n-3 polyunsaturated fatty acids) have been commonly associated with pregnancy hypertension by way of inflammation, oxidative stress, dyslipidaemia, altered vascular and fibrinolytic functions.[20, 21, 44] Therefore, a focus on the role of maternal adiposity and polyunsaturated fatty acids on blood pressures would be examined in this thesis and a brief review of literature would be presented accordingly in chapters 3 and 4.

Hypertension in pregnancy is a well-established risk factor for adverse outcomes in the offspring, such as small for gestational age and higher blood pressures.[20, 67] Particularly in Asian populations, these complications have large public health implications as the rate of small for gestational age[69, 70] and hypertension is highest in Asia.[71, 72] Although various risk factors have been associated with small for gestational age[69, 73] and higher blood pressures in the offspring,[65, 74] the focus in this thesis would be on the role of maternal blood pressures. A brief description on the current literature on the associations between maternal blood pressures and offspring size at birth and blood pressures would be presented accordingly in chapters 5 and 6.

1.4 Current Gaps in Epidemiological Study of Arterial Blood Pressures in Pregnant Women

Study on factors affecting maternal blood pressures during pregnancy is important as blood pressure is a known risk factor for cardiovascular risk in the women and her offspring later in life.[20, 67] The focus on lifestyle factors, particularly for adiposity[75-77] or diet,[78, 79] have been very well studied, and findings from these studies have led to the development of health recommendations or policy changes to reduce risk of hypertension and cardiovascular outcomes.[80, 81] Likewise for offspring health, pregnancy associated hypertension is strongly associated adverse outcomes such as low birth weight and hypertension in offspring later life. [65, 67, 74, 82]

However, the current literature in pregnant women is limited as maternal hypertension, instead of blood pressures are often examined, and few have concurrent examinations of maternal peripheral and central blood pressures. Moreover, the observed maternal blood pressure relations were based on studies that were conducted in predominantly white population and few were done in Asian population.

Because blood pressure has a normal distribution and is known to vary between populations due to geographic and environmental factors,[65] differences in the magnitude or threshold for the lifestyle factors and blood pressure relations are likely to exist. For example, the lower body mass index (BMI) cut-offs for obesity in relation to cardiovascular risk in Asians compared to non-Asians[83, 84] is a clear illustration that population differences exist.

Therefore, the present study is aimed to address the gaps identified from the review of literature. The study findings reported in this thesis will provide a deeper understanding on the blood pressure range and variation during pregnancy in relation to lifestyle factors and offspring outcomes. The study findings may also contribute to the health promotion and enable targeted monitoring or prevention programmes for Asian women and children.

1.5 Study Aims and Objectives

To address the gaps in the literature, the following studies are aimed to examine determinants and consequences of maternal blood pressures during pregnancy in an Asian birth cohort of Chinese, Malay and Indian women. The first aim of the study is to examine lifestyle modifiable factors that may be associated with blood pressures in pregnancy, and the specific study objectives are:

1. To examine the relationship between maternal obesity, gestational diabetes and blood pressures during pregnancy.
2. To examine the relationship between maternal plasma levels of polyunsaturated fatty acids and blood pressures during pregnancy.

The 2nd aim of the study is to examine the influence of maternal blood pressures during pregnancy on offspring, and the specific study objectives are:

1. To examine the relationship between maternal blood pressures during pregnancy and offspring size at birth.

2. To examine the relationship between maternal blood pressures and offspring blood pressures at 3 years old.

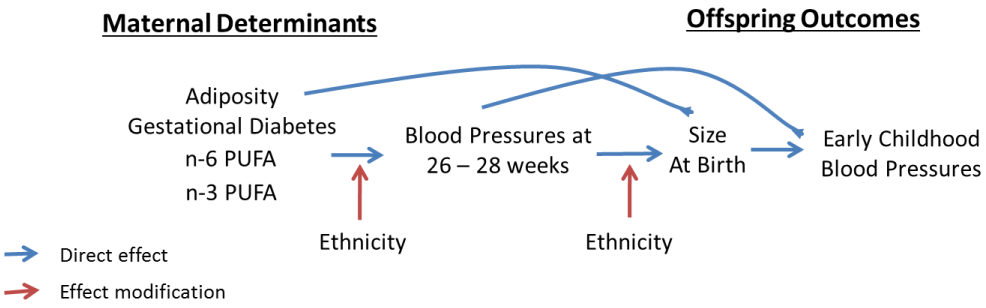

Figure 1-3. Conceptual Framework of Pathways for the Relations Between Maternal Adiposity, Plasma Polyunsaturated Fatty Acids (PUFA), Blood Pressures, Offspring Size at Birth and Blood Pressures at 3 Years Old

As maternal adiposity, gestational diabetes, plasma PUFAs and blood pressures may be inter-correlated, a conceptual framework was developed to examine their pathways with respect to the blood pressure relations examined in this thesis (Figure 1-3). Maternal adiposity, gestational diabetes, plasma PUFAs may affect blood pressures during pregnancy, and maternal blood pressures in turn, may affect offspring size at birth and blood pressures at 3 years old. Maternal adiposity and gestational diabetes may also affect offspring weight and gestation at birth. Maternal ethnicity may modify the maternal adiposity, gestational diabetes, plasma PUFAs and blood pressure relations; and between maternal blood pressures and offspring size at birth and blood pressures at 3 years old.

The study objectives would be addressed accordingly in the following chapters of this thesis. In Chapter 2, a general methodology on the overall study design, population and study measures would be presented. Chapters 3

and 4 would address the first aim of the study on the influence of maternal adiposity and polyunsaturated fatty acids (lifestyle modifiable factors) on maternal blood pressures during pregnancy. Chapters 5 and 6 would address the second aim of the study on the influence of maternal blood pressures on her offspring health in terms of weight at birth and subsequent blood pressures at 3 years old. Finally, in Chapters 7 and 8, the findings from the studies on lifestyle factors and offspring outcomes would be synthesized and discussed. An overall conclusion on the implications of these findings and future directions would be presented.

CHAPTER 2

GENERAL METHODS

2.1 Introduction

The **G**rowing **U**p in **S**ingapore **T**owards healthy **O**utcomes (GUSTO) is an ongoing prospective birth cohort study set up in 2009, with detailed follow-up of women and their offspring into early childhood.[85] The GUSTO study was primarily designed to study epigenetic markers, and early life factors and the developmental pathways to metabolic disorders and altered body composition. The GUSTO study was conducted in Singapore, situated at the north of the equator (between latitudes 1°09'N and 1°29'N and longitudes 103°36'E and 104°25'E), which is an urban city-state with a multi-ethnic society comprising predominantly of Chinese (76.2%), Malay (15.0%), Indian (7.4%) and others (1.4%).[86] The study was reviewed and approved by the SingHealth and National Health Group Institutional and Domain Specific Review Boards. Written consent was also obtained from all women participating in the study.

2.2 Study Design

2.2.1 Overview

The GUSTO study is a prospective birth cohort study that measures exposures from fetal life to early childhood of the offspring. The GUSTO study incorporates a comprehensive measure of various phenotypic and epigenetic observations in the early years of the offspring. The study is conducted in two institutions (KK Women's and Children's Hospital and National University Hospital) that provide tertiary maternal and childcare services, with close collaborations with various scientists and clinicians.

2.2.2 Inclusion and Exclusion Criteria

Between June 2009 and September 2010, pregnant women who were receiving antenatal care at KK Women's and Children's Hospital and National University Hospital at less than 14 weeks gestation were screened for study eligibility. Only women aged between 18 to 50 years old, citizens or permanent residents of Singapore with homogenous parental and spousal's ethnic background (Chinese, Malay or Indian) and intending to reside in Singapore for the next 5 years were eligible for study participation. Women who were on chemotherapy, psychotropic drugs, have type 1 diabetes or unwilling to donate their birth tissues (umbilical cord, cord blood and placenta) would be excluded from the study.

2.3 Study Population

2.3.1 Pregnant Women

From the 2034 eligible women who were screened at the two study sites, a total of 1162 women consented into the GUSTO main cohort. Briefly, the women in the main GUSTO cohort were enrolled into the study at a mean age 30.3 ± 5.2 years old and they were predominantly Chinese (54.3%), followed by Malays (27.4%) and Indians (18.3%) (Table 2-1). About 32.1% of the women had completed tertiary education or higher. Women in the GUSTO cohort tended to be non-smokers and non-drinkers. About 28% of GUSTO women engaged in moderate to strenuous activities.

2.3.2 Children

Among the 1162 women in the main GUSTO cohort, 1097 (94.4%) had complete birth information at delivery, of which, 1087 women had singletons and 10 had twins. There were similar proportions of male (52.5%) and female (47.5%) singleton offspring. The mean gestation and weight at birth for the singleton offspring were 38.6 ± 1.6 weeks and 3.1 ± 0.4 kg, respectively.

Table 2-1. Characteristics of GUSTO Study Participants

Characteristics	Women N=1162	Offspring N=1087
Age at enrolment (years)	30.3 ± 5.2	-
Ethnicity		
Chinese	630 (54.3%)	576 (55.1%)
Malay	318 (27.4%)	281 (26.9%)
Indian	212 (18.3%)	189 (18.1%)
Education		
Primary to Secondary	371 (32.4%)	
GCE/Vocational/Polytechnic	406 (35.5%)	
Tertiary and above	368 (32.1%)	
Parity		
Nulliparous	467 (42.9%)	
Mulitparous	620 (57.1%)	
Smoking history		
Non-smokers	941 (85.9%)	
Ever smokers	155 (14.4%)	
Alcohol intake		
None	691 (64.3%)	
Yes	383 (35.7%)	
Physical activity		
None to Light	809 (73.8%)	
Moderate to Strenuous	288 (26.2%)	
Gestation at Delivery, weeks	-	38.6 ± 1.6
Birth weight, Kg	-	3.1 ± 0.4
Offspring Sex		
Male	-	571 (52.5%)
Female	-	516 (47.4%)

Data are presented as mean ± SD or in column percentages (%)

The variables with missing information for women in the GUSTO study are: age (n=2), ethnicity (n=2), education (n=17), parity (n=75), smoking status (n=66), alcohol intake (17), physical activity (n=65).

Information on twin offspring is not included.

2.4 Data Collection in the GUSTO Study

The GUSTO study has incorporated a systematic and detailed collection of parental and offspring information from early pregnancy to early childhood of the offspring (Table 2-2). The information collected, is comprised of demographic, lifestyle, dietary, anthropometric, blood pressures, mental wellness and health outcomes such as hypertension and diabetes. A wide array of biological specimens from blood (maternal and cord blood), birth tissues (umbilical cord and placenta) and buccal smears (offspring) were collected. The following reflects a brief description of exposures, covariates and outcomes, which were examined in this thesis (Figure 2-1).

Table 2-2. Type and Sequence of Information Collected in the GUSTO Study

	Antenatal Visits			**Postnatal Visit**
	<14 weeks	**26 – 28 weeks**	**Delivery**	**36 months**
Women				
Questionnaires				
Demographic	*			
Socio-economic	*			
Smoking		*		
Alcohol		*		
Physical activity		*		
Dietary		*		
Mental wellness		*		
Medical history		*		
Obstetric history		*		
Medication	*	*		
Physical Examination				
Anthropometric		*		
Blood pressures		*		
Blood Biomarkers		*		
Glucose		*		
PUFA				
Pregnancy Outcomes			*	
Hypertension			*	
Preterm birth			*	
Medication				
Offspring				
Physical Measurements				
Anthropometric			*	*
Blood pressures				*

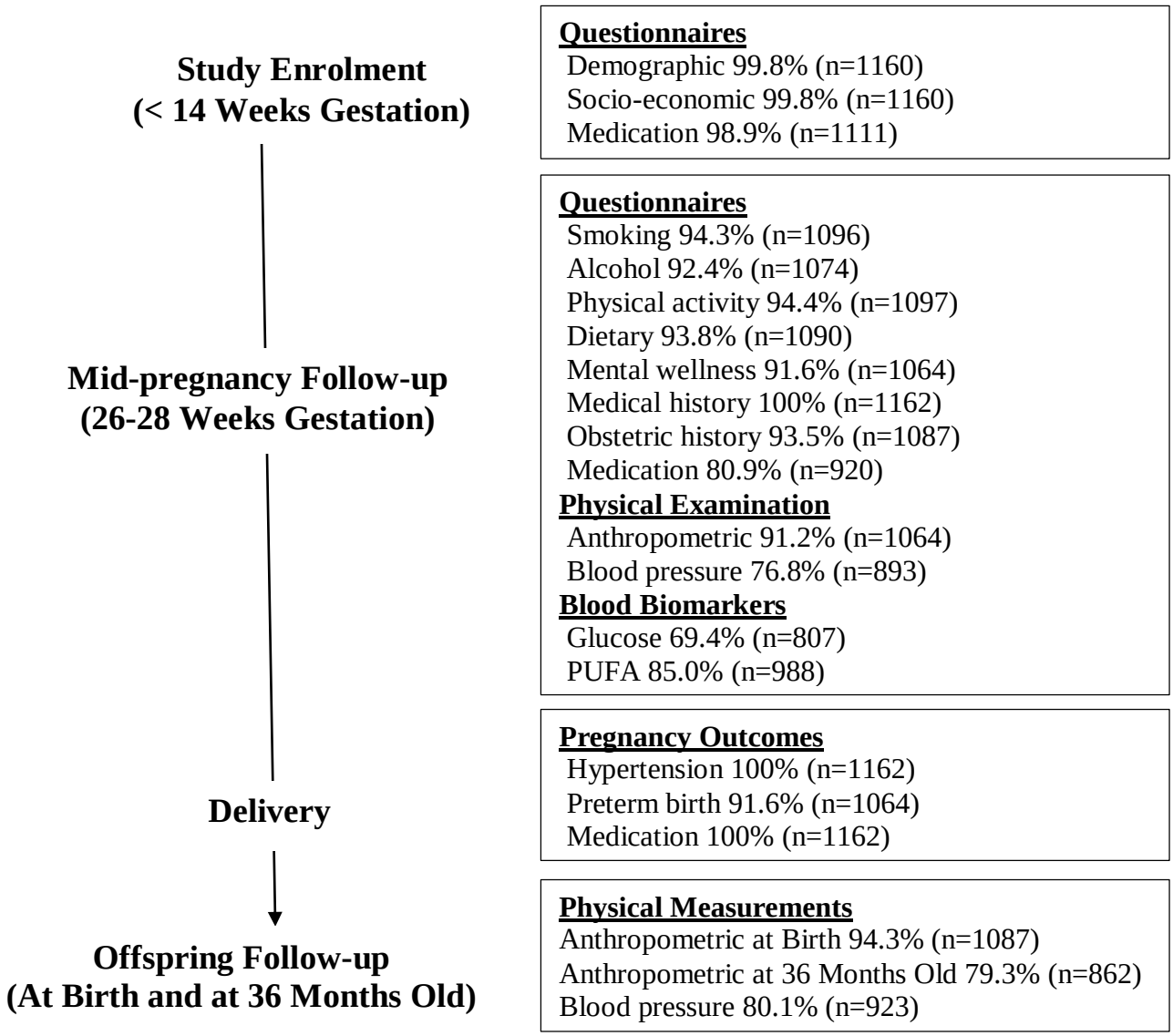

Figure 2-1. Response Rates for GUSTO Study Measures

2.4.1 Questionnaires

The women in this study were interviewed by trained research coordinators at each GUSTO visit in early pregnancy at study enrolment and at mid-pregnancy, between 26 – 28 weeks gestation. The questionnaires were designed to collect information on demographic, socio-economic, lifestyle, dietary, mental wellness, medical and obstetric history. The questionnaires were designed to collect information on demographic, socio-economic,

lifestyle, dietary, mental wellness, medical and obstetric history. In particular the women in the study were asked if they were ever diagnosed by a healthcare professional to have hypertension, diabetes, myopia. Information on antenatal use of medication or supplement were solicited from study participants at the first (<14 weeks gestation) and second (26-28 weeks gestation) GUSTO study visits. Overall, there were good responses (98.9% and 80.9%, respectively), and the information on medication collected during these study visits were on supplement use.

These questionnaires were forward and backward translated into Chinese, Malay and Indian languages (Appendices 1-3: Recruitment Visit 1 and 26- 28 Weeks GUSTO Visit Questionnaires; and Edinburgh Postnatal Depression Scale).

2.4.2 Physical Examinations

The physical examinations performed in the GUSTO study included anthropometric and blood pressures at the GUSTO mid-pregnancy study visit, between 26 – 28 weeks gestation. For the women, the anthropometric measures included were height, weight and skinfold thickness sites at biceps, triceps, supra-ailiac and subscapular (Appendix 4: Anthropometric Measurements for Participants [Parents and Children]). Peripheral blood pressures were taken from the brachial arm, and central blood pressures were estimated from radial pulse wave analysis (Appendix 5: Procedures for using BPro and A-PULSE CASP). Similarly, offspring height, weight and blood pressures were taken at GUSTO visit at the age of 3 years old (Appendix 4

and 6: Measuring Blood pressures by Dynamap CARESCAPETM V100 [General Instruction for Mother and Child]).

2.4.3 Blood Biomarkers

Maternal blood biomarkers were collected at GUSTO mid-pregnancy visit between 26 – 28 weeks gestation, in which plasma n-3 and n-6 polyunsaturated fatty acids (PUFAs) from the fasting blood specimen, and glucose levels at fasting and 2-hour were determined.

2.4.4 Pregnancy and Birth Outcomes

Information on the offspring's birth measurements and pregnancy complications were abstracted from medical records. The birth measures abstracted were offspring gestational age, birth weight, length and head circumference and placental weight. Other information included in the data abstraction was obstetrician's diagnosis of preeclampsia, gestational hypertension and preterm birth. Information on medications prescribed during the intrapartum stage were also abstracted from medical records after delivery, and they include the use of intravenous antibiotics, intra-muscular steroids, labour analgesics and analgesia

CHAPTER 3

MATERNAL OBESITY, GESTATIONAL DIABETES AND BLOOD PRESSURES DURING PREGNANCY IN SOUTH-EAST ASIAN WOMEN

This chapter was based on the paper published in J Hypertension:

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3.1 Introduction

High blood pressure affects 10-12% of pregnancies and it is associated with adverse pregnancy outcomes including eclampsia, preterm birth and caesarean delivery.[20, 22] The pathophysiology of hypertension in pregnancy is unclear, and a number of risk factors have been proposed, including older maternal age, smoking, nulliparity, previous abortions, multiple pregnancy, and obesity. [67, 87] Previous studies have mainly focused on the relation between obesity and peripheral blood pressures in pregnancy (Table 3-1), [30, 77, 88-93] but less is known for central blood pressures, which is of interest because they reflect different cardiovascular adaptations compared to peripheral pressures. [94, 95]

Meanwhile, most studies of the obesity-blood pressure relation have been conducted in Caucasians, and studies in Asian women and particularly pregnant women are very limited. Furthermore, no study has specifically assessed differences in the obesity-blood pressure relation within Asian pregnant women of different ethnicities. The three major Asian ethnic groups, Chinese, Indian, and Malay, comprise of more than 43% of the total global population, and obesity has increasingly become a major public health problem in the three ethnicities,[96, 97] with important implications for adverse pregnancy outcomes in the populations and potential long-term impact on the mothers and their offspring.[75, 98] Some studies suggest that there are ethnic differences in predisposition to obesity within Asians,[99] and obesity may be differentially related to insulin resistance and inflammatory markers among Chinese, Malays and Indians.[100]

Table 3-1. Evidence Table for the Association between Maternal Body Mass Index (BMI) and Blood Pressures

ID	Author /Year	Study Design	Sample size/ Country	Gestational Age at Blood Pressure Measurements	Measures of Blood Pressures	Results
1	Grinheim et al, 2012	Prospective cohort	57/Norway	At 14-16 weeks; 22-24; 30-32; 36 weeks and 6 months postnatal	Peripheral SBP, DBP and MAP	Pre-pregnant overweight women had higher SBP, DBP and MAP than those with normal pre-pregnant weight SBP: -6.0 (95%CI -11.4 to -0.5) mmHg; DBP: -4.7 (95%CI -8.8 to -0.6) mmHg; MAP: -4.8 (-95%CI -9.4 to -0.2) mmHg
2	Fujime et al / 2012	Prospective cohort	830/ Healthy pregnant women/ Japan	12 – 36 weeks	Central SBP	Maternal BMI was correlated with central SBP (Pearson r=0.30; p<0.001)
3	Gaillard et al/ 2011	Prospective cohort	6902/ Netherlands	From 1st trimester onwards till 3rd trimester	Peripheral SBP and DBP	Prepregnancy obesity is associated with blood pressures (SBP & DBP) in all pregnancy trimesters. SBP: 1.03 (95%CI 0.95 to 1.10) mmHg in 1st trimester; 0.98 (95%CI 0.91 to 1.04) mmHg in 2nd trimester; 0.89 (95%CI 0.83 to 0.96) mmHg in 3rd trimester.

						DBP: 0.83 (95%CI 0.77 to 0.88) mmHg in 1st trimester; 0.81 (95%CI 0.76 to 0.86) mmHg in 2nd trimester; 0.74 (95%CI 0.69 to 0.79) mmHg in 3rd trimester.
4	Teng et al/ 2010	Prospective cohort	600/ China	From 1st trimester onwards till 3rd trimester	Peripheral SBP and DBP	Overweight/ obese pre-pregnant women had higher SBP and DBP than pre-pregnant normal weight women. Overweight vs normal weight: 9.9 mmHg (SBP); 7.5 mmHg (DBP); Obese vs normal weight: 14.3 mmHg (SBP) and 7.9 mmHg (DBP)
5	Thompson et al/ 2009	Prospective cohort	1733/ Sweden & USA	From 1st trimester onwards till 3rd trimester	Peripheral SBP and DBP	At any gestational age, mean SBP (or DBP) increased with increasing pre-pregnancy BMI; the increase however is attenuated with increasing pre-pregnancy BMI, particularly later in pregnancy
6	Strevens et al/ 2002	Prospective cohort	166/ Sweden	From 1st trimester onwards till 3rd trimester	Peripheral SBP and DBP	Baseline (early pregnancy) BMI influenced third trimester SBP significantly; but DBP levels were only significant in primiparous women only

7	Tomoda et al/ 1996	Prospective cohort	2349/ Japan	From 1st trimester onwards till 3rd trimester	MAP	Women with early pregnancy obesity had higher MAP than normal weight women Primiparous women: 81.4(10.8) vs 88.7(12.5) mmHg at 39 weeks Multiparous women: 79.9(9.2) vs 84.3(10.0) mmHg at 39 weeks

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; BMI, body mass index

Therefore, we examined the association of maternal adiposity with peripheral and central blood pressures as measures of arterial compliance in a large cohort of South Asian pregnant women in Singapore. Since the cohort members were from three major ethnic groups (Chinese, Malay and Indian), we also aimed to investigate whether the associations were modified by ethnicity. Lastly, as obesity and gestational diabetes are prevalent in Asian women,[101] we assessed their independent and joint associations with blood pressures across ethnicities of South Asian women.

3.2 Methods

Study Participants

The study subjects were drawn from the **G**rowing **U**p in **S**ingapore **T**owards healthy **O**utcomes (GUSTO) cohort study,[85] which comprised of 1162 pregnant women recruited from KK Women's and Children's Hospital (KKH) and National University Hospital (NUH) during 2009 and 2010. We recruited pregnant women who were Singapore citizens or permanent residents, and who conceived naturally and intended to deliver at the two hospitals. We only included pregnant women who were Chinese, Malay or Indian with homogenous parental ethnic background. Women with type 1 diabetes, on chemotherapy or psychotropic drugs were excluded. Among the 893 (76.8%) women who attended the GUSTO 2nd trimester study visit for blood pressures and radial pulse waves measurement, 64 women were excluded due to poor measurement of radial pulse wave forms and 30 women due to missing information on age, weight or height measurements. Therefore, data from 799

(89.5%) women were available for analysis (Figure 3-1). No significant differences in age and body mass index were found between women who were included and excluded from analysis because of poor measurement of radial pulse wave forms (Supplementary Table 3-1). The GUSTO study was approved by the SingHealth and National Health Group Institutional and Domain Specific Review Boards.

Blood Pressure Measurement

The blood pressures and anthropometric measurements were performed at mean 27 ± 1.2 weeks gestation during the GUSTO 2nd trimester study visit. Research coordinators were trained prior to commencement of fieldwork, and standard operating procedures were adopted. Participants were required to abstain from caffeine intake for at least 30 minutes prior to blood pressure measurement. Brachial systolic (SBP) and diastolic (DBP) pressures were measured thrice at 30–60 seconds intervals with an oscillometric device MC3100 (HealthSTATS International Pte Ltd, Singapore). We applied the A-pulse tonometer (BPro®, HealthSTATS International Pte Ltd, Singapore) on the radial artery of the same arm, for continuous sampling of radial artery waveforms over 1 minute following standard procedures.[12, 102] These waveforms were calibrated with the averaged brachial pressures to derive the central SBP. Central pulse pressure (PP) was calculated based on the difference between CASP and DBP.[62] The BPro® device has shown high agreement and correlation (r^2=0.98) with invasive central aortic pressures measurements.[12, 102]

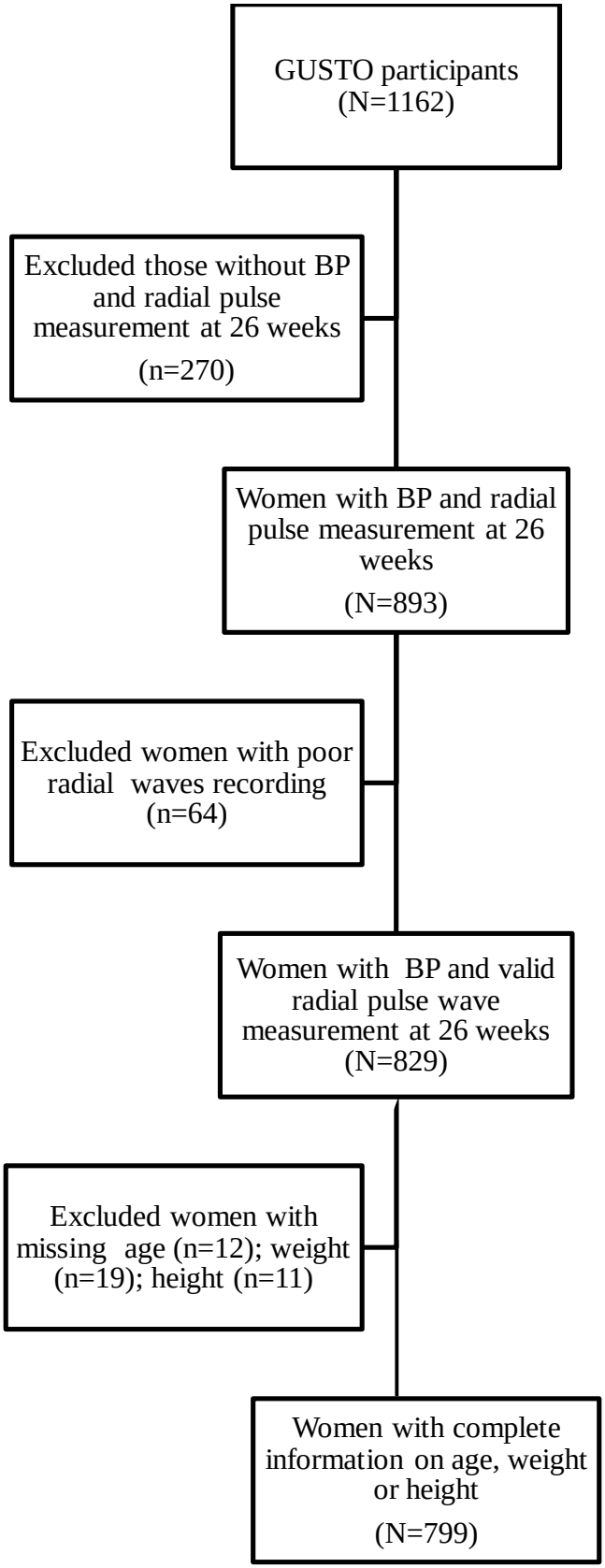


Figure 3-1. Flow Chart of the GUSTO Study Sample Selected for Analysis

Adiposity Measures (Body Mass Index and Skinfold Thickness)

Maternal height and weight were measured during the 2nd trimester study visit. Height was measured twice to the nearest 0.1 cm, barefooted in the horizontal Frankfort plane using a Seca 213 Portable Stadiometer (SECA, Hamburg, Germany). Weight was measured to the nearest 0.1 kg using a calibrated weighing scale (SECA 803 electronic flat scale: SECA, Hamburg, Germany). Measurements were repeated if readings differed by more than 1 cm and 0.2 kg respectively, and the average of all 3 readings were used. Body mass index (BMI, kg/m^2) was calculated as weight in kg divided by square of height in meters. Skinfold thicknesses were measured at 4 sites (biceps, triceps, subscapular and suprailiac) following standard procedures[103] using Holtain Tanner/Whitehouse skinfold calipers (Holtain Ltd, Crymych, United Kingdom). All measurements were made in triplicate to nearest 0.2 mm and the readings were averaged. The sum of skinfold thickness was derived by summating the averaged skinfold thicknesses of the 4 sites.

Covariates

Established risk factors for hypertension were assessed by questionnaire as potential confounders: maternal age, ethnicity, education, parity and pre-existing chronic hypertension. Glycaemic status in pregnancy was ascertained using a 75mg oral glucose tolerance test performed at the same study visit; gestational diabetes was defined as fasting plasma glucose ≥7.0 mmol/l or 2 hour glucose ≥11.1 mmol/l according to the World Health Organization (WHO) classification.[104]

Data Analysis

Maternal BMI was categorized according to the WHO international classification[105]: underweight (<18.5 kg/m^2), normal weight (18.5 to 24.9 kg/m^2), overweight (25.0 to 29.9 kg/m^2) and obesity (≥30.0 kg/m^2); and Asian classification [84]: BMI <18.5, 18.5 to 22.9, 23.0 to 27.4, and ≥27.5 kg/m^2, respectively. Because there were only 6 underweight women during the study visit and their BMIs were between 17.9 and 18.5 kg/m^2, they were combined into the normal weight group. The sum of skinfold thickness was analysed as continuous and categorical (tertiles) variables. The unadjusted means and proportions of maternal characteristics according to BMI categories were compared using ANOVA and chi-square test for continuous and categorical variables respectively.

We performed multivariate linear regression model to examine the independent relations of BMI and sum of skinfold thickness with peripheral and central blood pressures, adjusting for maternal age, ethnicity (Chinese, Malay, Indian), education (primary school or less, secondary school, tertiary school or more), parity (no, one, two or more live-births), current smoking status (yes, no), gestational diabetes (yes, no), maternal height and heart rate. Only 16 women had pre-existing chronic hypertension, so this variable was not included as a covariate due to problems with model convergence. A sensitivity analysis in women without pre-existing chronic hypertension revealed similar results (data not shown).

Effect modification by ethnicity (Chinese, Malay, Indian) and gestational diabetes (yes, no) was evaluated from the multiplicative interaction term

between continuous BMI and the effect modifier added to the main effect model, and results were then stratified by ethnicity and gestational diabetes. We further explored these associations by joint stratification of ethnicity and gestational diabetes to assess their collective influences on the association between BMI and blood pressure outcomes. All tests performed were two-tailed and statistical significance was set at 0.05 using Stata version 11.2 (Statacorp, College Station, Texas).

3.3 Results

The mean BMI of the 799 women was 26.24 ± 4.44 kg/m^2; 35% were overweight and 19% were obese (Table 3-2). Compared with normal weight women, obese women were more likely to have pre-existing chronic hypertension and gestational diabetes. The prevalence of obesity was higher in Malay women and those with low educational attainment. There were no differences in age and smoking status among obesity categories. Compared to normal weight women, obese women had higher skinfold thickness, pulse rate and the four blood pressure measures.

Table 3-2. Maternal Characteristics by Categories of Body Mass Index (BMI) at 2nd Trimester[a]

Maternal Characteristics	Overall	2nd Trimester Body Mass Index categories			
		Normal Weight (≤24.9 kg/m^2)	Overweight (25.0-29.9 kg/m^2)	Obese (≥30.0 kg/m^2)	*P*
n	799	368	279	152	
Age (years)	30.44 ± 5.16	30.29 ± 5.03	30.54 ± 5.32	30.59 ± 5.18	0.77
Ethnicity					<0.001
Chinese	435 (54.4%)	243 (66.0%)	149 (53.4%)	43 (28.3%)	
Malay	229 (28.7%)	75 (20.4%)	78 (27.9%)	76 (50.0%)	
Indian	135 (16.9%)	50 (13.6%)	52 (18.7%)	33 (21.7%)	
Education					<0.001
Primary to secondary	256 (32.4%)	113 (31.2%)	87 (31.3%)	56 (37.6%)	
GCE/Vocational/Polytechnic	276 (34.9%)	103 (28.4%)	112 (40.3%)	61 (40.9%)	
Tertiary	257 (32.6%)	146 (40.3%)	79 (28.4%)	32 (21.5%)	
Parity					<0.001
Nulliparous	329 (42.9%)	170 (48.3%)	110 (41.0%)	49 (33.3%)	
Multiparous	438 (57.1%)	182 (51.7%)	158 (59.0%)	98 (66.7%)	
Smoking history					0.47
Never smokers	673 (86.7%)	310 (86.8%)	244 (88.1%)	119 (83.8%)	
Ever smokers	103 (13.3%)	47 (13.2%)	33 (11.9%)	23 (16.2%)	
Chronic hypertension					<0.001
No	773 (96.7%)	361 (99.7%)	275 (98.9%)	137 (91.9%)	
Yes	16 (3.3%)	1 (0.3%)	3 (1.1%)	12 (8.05%)	
Gestational diabetes					0.01
No	602 (81.6%)	292 (86.1%)	205 (78.2%)	105 (76.6%)	
Yes	136 (18.4%)	47 (13.9%)	57 (21.8%)	32 (23.4%)	
Sum of skinfold Thickness (mm)	79.95 (19.63)	66.71 (13.35)	85.04 (14.51)	102.72 (14.83)	<0.001
Peripheral BP (mmHg)					
SBP	109.48 ± 11.21	104.62 ± 9.93	111.34 ± 9.92	117.82 ± 10.46	<0.001
DBP	66.82 ± 8.45	63.81 ± 7.59	67.49 ± 7.87	72.93 ± 7.92	<0.001
Central BP (mmHg)					
SBP	96.89 ± 10.09	92.81 ± 9.19	98.45 ± 8.97	103.89 ± 9.54	<0.001
PP	30.06 ± 6.52	29.00 ± 6.29	30.96 ± 6.61	30.95 ± 6.57	<0.001
Pulse rate (beats/minute)	104.01 ± 16.26	101.09 ± 15.96	104.20 ± 15.28	110.72 ± 16.80	<0.001

BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

[a] Data are represented as n (%) or mean ± SD where appropriate.

P values derived from chi-squared tests for categorical variables and ANOVA for continuous variables.

Missing values for variables: Education = 10; Parity = 32; Smoking history = 23; Gestational diabetes mellitus = 61 and Sum of skinfold thicknesses = 2.

In a multivariable linear regression model, 2nd trimester BMI was significantly associated with all blood pressure outcomes (all $P<0.001$; Table 3-3): the increases in blood pressures (mmHg) for each unit (kg/m^2) increase in BMI were 1.19 [95% confidence interval (CI) 1.03-1.36] for peripheral SBP, 0.76 (95% CI 0.63-0.89) for peripheral DBP, 1.02 (95% CI 0.87-1.17) for central SBP and 0.26 (95% CI 0.16-0.37) for central PP. When we stratified BMI to three categories according to WHO international classification, overweight and obese women had significantly greater blood pressures compared with normal weight women. Similar trends were observed when maternal BMI was categorized according to the Asian classification (Supplemental Table 3-2). The results remained unchanged when we excluded the 6 underweight women from the analysis (data not shown). When using skinfold thickness as the adiposity measure, positive associations were observed with all four blood pressure outcomes (all $P<0.001$; Table 3-3). For example, each 10 mm increase in sum of skinfold thickness was associated with 2.17, 1.33, 2.03, and 0.69 mmHg higher peripheral SBP and DBP, central SBP and PP, respectively.

We observed a significant interaction between BMI and ethnicity in relation to central PP ($P = 0.03$ for interaction; Table 3-4): each kg/m^2 increase in BMI was associated with 0.50 (95% CI 0.33-0.68) mmHg higher central PP in Chinese, while the corresponding point estimate was 0.15 (95% CI -0.01 to 0.30) in Malays and 0.06 (95% CI -0.21 to 0.32) in Indians. The relations of BMI with peripheral and central SBP were generally stronger in Chinese compared to Malays or Indians, although the interaction tests were not statistically significant. Similar patterns were observed when using

skinfold thickness as adiposity measure, but the interaction tests with ethnicity were not statistically significant. For example, each 10 mm increase in sum of skinfold thicknesses was associated with 1.01 (95% CI 0.67-1.35) mmHg higher central PP in Chinese, compared with 0.43 (95% CI 0.06-0.80) mmHg in Malays and 0.41 (95% CI -0.17 to 0.99) mmHg in Indians.

We also found significant interactions between adiposity measures and gestational diabetes in relation to peripheral DBP and central SBP, with stronger associations in women with gestational diabetes compared to those without (both $P<0.05$ for interaction; Table 3-5). Interaction tests for peripheral SBP or central PP were not statistically significant, but trends for stronger associations were generally observed in women with gestational diabetes compared with normoglycaemic women.

In the analysis jointly stratified by ethnicity and gestational diabetes, we found that the associations between BMI and blood pressure outcomes were strongest in Chinese women with gestational diabetes (Table 3-6). When stratified by ethnicity, interaction tests between BMI and gestational diabetes in relation to blood pressures (peripheral SBP, DBP and central SBP) were significant in Chinese women, but not in Malay or Indian women. Similarly, when stratified by gestational diabetes, interaction tests between BMI and ethnicity in relation to blood pressures (central SBP and PP) were significant in women with gestational diabetes, but not in those without gestational diabetes.

Table 3-3. Associations between 2nd Trimester Obesity Measures and Blood Pressures[a]

		Peripheral Blood Pressures (mmHg)				Central Blood Pressures (mmHg)			
		SBP		DBP		SBP		PP	
	N	ß (95% CI)	*P*	ß (95% CI)	*P*	ß (95% CI)	*P*	ß (95% CI)	*P*
2nd Trimester BMI (kg/m^2)	799	1.19 (1.03 to 1.36)	<0.001	0.76 (0.63 to 0.89)	<0.001	1.02 (0.87 to 1.17)	<0.001	0.26 (0.16 to 0.37)	<0.001
2nd Trimester BMI (categories)									
Normal weight (≤24.9 kg/m^2)	368	Reference		Reference		Reference		Reference	
Overweight (25.0-29.9 kg/m^2)	279	6.18 (4.60 to 7.76)	<0.001	3.12 (1.91 to 4.33)	<0.001	5.25 (3.79 to 6.71)	<0.001	2.13 (1.14 to 3.12)	<0.001
Obese (≥30.0 kg/m^2)	152	12.95 (10.94 to 14.95)	<0.001	8.58 (7.04 to 10.11)	<0.001	11.18 (9.33 to 13.02)	<0.001	2.59 (1.34 to 3.86)	<0.001
Sum of skinfold thickness (per 10 mm increase)	797	2.17 (1.79 to 2.54)	<0.001	1.33 (1.05 to 1.62)	<0.001	2.03 (1.69 to 2.36)	<0.001	0.69 (0.46 to 0.92)	<0.001
Sum of skinfold thickness (categories)									
First tertile (28.3-69.9 mm)	271	Reference		Reference		Reference		Reference	
Second tertile (70.0-87.2 mm)	252	2.99 (1.21 to 4.78)	0.001	1.52 (0.17 to 2.88)	0.028	2.99 (1.38 to 4.61)	<0.001	1.47 (0.41 to 2.54)	0.007
Third tertile (87.3-138.3 mm)	274	8.92 (7.08 to 10.76)	<0.001	5.48 (4.09 to 6.88)	<0.001	8.56 (6.89 to 10.24)	<0.001	3.08 (1.98 to 4.18)	<0.001

BMI, body mass index; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure

[a] The results were derived from multiple linear regression models with adjustment for maternal age, ethnicity, education level, parity status, smoking history, gestational diabetes, body height and heart rate.

Table 3-4. Associations between 2nd Trimester Obesity Measures and Blood Pressures: Stratified by Maternal Ethnicity[a]

		Peripheral Blood Pressures (mmHg)				Central Blood Pressures (mmHg)			
		SBP		DBP		SBP		PP	
	N	ß (95% CI)	*P*	ß (95% CI)	*P*	ß (95% CI)	*P*	ß (95% CI)	*P*
2nd Trimester BMI in Chinese women (kg/m^2)	435	1.45 (1.16 to 1.74)	<0.001	0.76 (0.53 to 0.98)	<0.001	1.26 (0.99 to 1.53)	<0.001	0.50 (0.33 to 0.68)	<0.001
2nd Trimester BMI category in Chinese women									
Normal weight (≤24.9 kg/m^2)	243	Reference		Reference		Reference		Reference	
Overweight (25.0-29.9 kg/m^2)	149	6.09 (3.96 to 8.23)	<0.001	2.92 (1.29 to 4.57)	<0.001	5.26 (3.29 to 7.24)	<0.001	2.33 (1.04 to 3.62)	<0.001
Obese (≥30.0 kg/m^2)	43	14.49 (10.99 to 17.99)	<0.001	8.14 (5.46 to 10.81)	<0.001	12.68 (9.44 to 15.91)	<0.001	4.54 (2.43 to 6.65)	<0.001
2nd Trimester BMI in Malay women (kg/m^2)	229	0.98 (0.74 to 0.42)	<0.001	0.75 (0.56 to 0.94)	<0.001	0.89 (0.67 to 1.12)	<0.001	0.15 (-0.01 to 0.30)	0.06
2nd Trimester BMI category in Malay women									
Normal weight (≤24.9 kg/m^2)	75	Reference		Reference		Reference		Reference	
Overweight (25.0-29.9 kg/m^2)	78	6.11 (2.92 to 9.30)	<0.001	3.68 (1.25 to 6.11)	0.003	5.17 (2.26 to 8.08)	0.001	1.49 (-0.46 to 3.45)	0.13
Obese (≥30.0 kg/m^2)	76	12.06 (8.79 to 15.33)	<0.001	9.13 (6.64 to 11.62)	<0.001	11.07 (8.09 to 14.05)	<0.001	1.94 (-0.06 to 3.94)	0.06
2nd Trimester BMI in Indian women (kg/m^2)	135	1.10 (0.73 to 1.48)	<0.001	0.79 (0.50 to 1.08)	<0.001	0.85 (0.51 to 1.19)	<0.001	0.06 (-0.21 to 0.32)	0.66
2nd Trimester BMI category in Indian women									
Normal weight (≤24.9 kg/m^2)	50	Reference		Reference		Reference		Reference	
Overweight (25.0-29.9 kg/m^2)	52	6.79 (2.84 to 10.73)	0.001	3.48 (0.42 to 6.64)	0.03	6.22 (2.65 to 9.78)	0.001	2.74 (0.03 to 5.45)	0.048
Obese (≥30.0 kg/m^2)	33	11.25 (6.83 to 15.67)	<0.001	8.28 (4.85 to 11.69)	<0.001	8.58 (4.59 to 12.58)	<0.001	0.31 (-2.72 to 3.34)	0.84
P for interaction (Ethnicity*BMI at 26 weeks)			0.17		0.92		0.31		0.03
Sum of skinfold thicknesses in Chinese women (per 10 mm increase)	433	2.36 (1.78 to 2.94)	<0.001	1.18 (0.74 to 1.62)	<0.001	2.19 (1.66 to 2.72)	<0.001	1.01 (0.67 to 1.35)	<0.001
Sum of skinfold thicknesses in Chinese women									
First tertile (28.3-69.9 mm)	170	Reference		Reference		Reference		Reference	

Second tertile (70.0-87.2 mm)	154	2.36 (0.01 to 4.69)	0.049	1.44 (-0.34 to 3.21)	0.11	2.95 (0.81 to 5.09)	0.007	1.51 (0.16 to 2.87)	0.03
Third tertile (87.3-138.3 mm)	109	9.18 (6.45 to 11.89)	<0.001	4.14 (2.08 to 6.19)	<0.001	8.72 (6.23 to 11.20)	<0.001	4.58 (3.00 to 6.15)	<0.001
Sum of skinfold thicknesses in Malay women (per 10 mm increase)	229	1.87 (1.25 to 2.49)	<0.001	1.44 (0.97 to 1.92)	<0.001	1.88 (1.32 to 2.44)	<0.001	0.43 (0.06 to 0.80)	0.02
Sum of skinfold thicknesses in Malay women									
First tertile (28.3-69.9 mm)	63	Reference		Reference		Reference		Reference	
Second tertile (70.0-87.2 mm)	59	6.08 (2.38 to 9.78)	0.001	4.09 (1.29 to 6.91)	0.004	6.30 (2.98 to 9.62)	<0.001	2.20 (0.05 to 4.35)	0.045
Third tertile (87.3-138.3 mm)	107	8.79 (5.49 to 12.10)	<0.001	6.89 (4.38 to 9.40)	<0.001	8.86 (5.89 to 11.82)	<0.001	1.96 (0.05 to 3.88)	0.045
Sum of skinfold thicknesses in Indian women (per 10 mm increase)	135	2.12 (1.27 to 2.97)	<0.001	1.43 (0.77 to 2.09)	<0.001	1.84 (1.08 to 2.59)	<0.001	0.41 (-0.17 to 0.99)	0.16
Sum of skinfold thicknesses in Indian women									
First tertile (28.3-69.9 mm)	38	Reference		Reference		Reference		Reference	
Second tertile (70.0-87.2 mm)	39	2.31 (-2.30 to 6.92)	0.32	-0.94 (-4.32 to 2.44)	0.58	0.12 (-3.84 to 4.09)	0.95	1.06 (-2.06 to 4.19)	0.50
Third tertile (87.3-138.3 mm)	58	9.03 (4.71 to 13.35)	<0.001	6.98 (3.81 to 10.15)	<0.001	8.38 (4.66 to 12.09)	<0.001	1.39 (-1.53 to 4.33)	0.35
P for interaction (Ethnicity*Sum of skinfold thickness)			0.79		0.52		0.96		0.17

BMI, body mass index; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure

[a] The results were derived from multiple linear regression models with adjustment for maternal age, education level, parity status, smoking history, gestational diabetes, body height and heart rate.

Table 3-5. Associations between 2nd Trimester Obesity Measures and Blood Pressures: Stratified by Glycaemic Status[a]

		Peripheral Blood Pressures (mmHg)				Central Blood Pressures (mmHg)			
		SBP		DBP		SBP		PP	
	N	ß (95% CI)	*P*	ß (95% CI)	*P*	ß (95% CI)	*P*	ß (95% CI)	*P*
2nd Trimester BMI in normoglycaemic women (kg/m^2)	602	1.16 (0.98 to 1.34)	<0.001	0.70 (0.56 to 0.85)	<0.001	0.98 (0.81 to 1.15)	<0.001	0.27 (0.16 to 0.39)	<0.001
2nd Trimester BMI category in n ormoglycaemic women									
Normal weight (≤24.9 kg/m^2)	292	Reference		Reference		Reference		Reference	
Overweight (25.0-29.9 kg/m^2)	205	5.43 (3.67 to 7.18)	<0.001	2.73 (1.38 to 4.07)	<0.001	4.46 (2.85 to 6.07)	<0.001	1.73 (0.65 to 2.82)	0.002
Obese (≥30.0 kg/m^2)	105	12.82 (10.51 to 15.12)	<0.001	8.21 (6.44 to 9.97)	<0.001	10.99 (8.89 to 13.11)	<0.001	2.79 (1.36 to 4.22)	<0.001
2nd Trimester BMI in GDM women (kg/m^2)	136	1.31 (0.86 to 1.762)	<0.001	1.04 (0.71 to 1.38)	<0.001	1.19 (0.76 to 1.62)	<0.001	0.15 (-0.13 to 0.43)	0.29
2nd Trimester BMI category in GDM women									
Normal weight (≤24.9 kg/m^2)	47	Reference		Reference		Reference		Reference	
Overweight (25.0-29.9 kg/m^2)	57	10.47 (6.43 to 14.51)	<0.001	5.98 (2.91 to 9.06)	<0.001	9.44 (5.62 to 13.27)	<0.001	3.46 (0.93 to 5.99)	0.008
Obese (≥30.0 kg/m^2)	32	14.85 (9.87 to 19.82)	<0.001	11.65 (7.87 to 15.43)	<0.001	13.82 (9.11 to 18.53)	<0.001	2.17 (-0.95 to 5.29)	0.17
P for interaction (GDM*BMI at 26 weeks)			0.21		0.030		0.046		0.77
Sum of skinfold thicknesses in normoglycaemic women (per 10 mm increase)	601	1.98 (1.56 to 2.39)	<0.001	1.14 (0.82 to 1.46)	<0.001	1.84 (1.47 to 2.22)	<0.001	0.70 (0.45 to 0.95)	<0.001
Sum of skinfold thickness in normoglycaemic women									
First tertile (28.3-69.9 mm)	222	Reference		Reference		Reference		Reference	
Second tertile (70.0-87.2 mm)	193	2.65 (0.69 to 4.61)	0.008	0.93 (-0.55 to 2.42)	0.22	2.59 (0.83 to 4.35)	0.004	1.66 (0.51 to 2.81)	0.005
Third tertile (87.3-138.3 mm)	186	8.31 (6.24 to 10.38)	<0.001	4.69 (3.13 to 6.27)	<0.001	7.89 (6.02 to 9.75)	<0.001	3.19 (1.98 to 4.41)	<0.001

Sum of skinfold thickness in GDM women (per 10 mm increase)	135	2.70 (1.61 to 3.79)	<0.001	1.96 (1.14 to 2.77)	<0.001	2.73 (1.72 to 3.73)	<0.001	0.77 (0.12 to 1.42)	0.020
Sum of skinfold thickness in GDM women									
First tertile (28.3-69.9 mm)	30	Reference		Reference		Reference		Reference	
Second tertile (70.0-87.2 mm)	42	1.51 (-3.85 to 6.88)	0.58	2.09 (-1.87 to 6.08)	0.29	2.53 (-2.42 to 7.47)	0.31	0.42 (-2.71 to 3.57)	0.79
Third tertile (87.3-138.3 mm)	63	9.36 (4.29 to 14.44)	<0.001	7.40 (3.64 to 11.16)	<0.001	10.12 (5.45 to 14.79)	<0.001	2.72 (-0.24 to 5.69)	0.072
P for interaction (GDM*Sum of skinfold thickness)			0.098		0.031		0.010		0.26

BMI, body mass index; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure

[a] A total of 61 women were excluded from the analysis because of missing information on gestational diabetes status. The results were derived from multiple linear regression models with adjustment for maternal age, ethnicity, education level, parity status, smoking history, body height and heart rate.

Table 3-6. Association between 2nd Trimester Obesity Measures and Blood Pressures: Joint Stratification by Maternal Ethnicity and Glycaemic Status[a]

	Peripheral Blood Pressures (mmHg)						Central Blood Pressures (mmHg)					
	SBP			DBP			SBP			PP		
	Normo-glycaemic	GDM	*P*[b]	Normo-glycaemic	GDM	*P*[b]	Normo-glycaemic	GDM	*P*[b]	Normo-glycaemic	GDM	*P*[b]
BMI												
Chinese	1.32 (0.97 to 1.66)	1.87 (1.23 to 2.52)	0.05	0.63 (0.36 to 0.89)	1.14 (0.66 to 1.63)	0.02	1.07 (0.75 to 1.39)	1.83 (1.22 to 2.43)	0.003	0.44 (0.24 to 0.65)	0.68 (0.28 to 1.08)	0.13
Malay	1.03 (0.76 to 1.3)	0.55 (-0.80 to 1.91)	0.66	0.75 (0.54 to 0.95)	0.88 (-0.29 to 2.05)	0.54	0.98 (0.73 to 1.22)	0.42 (-0.75 to 1.59)	0.31	0.23 (0.06 to 0.40)	-0.46 (-1.22 to 0.31)	0.02
Indian	1.12 (0.70 to 1.54)	0.66 (-0.96 to 2.28)	0.67	0.69 (0.38 to 1.01)	0.53 (-0.93 to 1.99)	0.49	0.80 (0.46 to 1.15)	0.55 (-1.25 to 2.35)	0.95	0.11 (-0.19 to 0.39)	0.02 (-0.97 to 1.00)	0.39
P[c]	0.62	0.09		0.65	0.65		0.93	0.02		0.32	0.01	
Skinfold thickness												
Chinese	2.07 (1.40 to 2.74)	2.59 (1.12 to 4.06)	0.18	0.87 (0.36 to 1.38)	1.61 (0.58 to 2.64)	0.055	1.87 (1.26 to 2.48)	2.76 (1.41 to 4.10)	0.03	1.00 (0.61 to 1.39)	1.15 (0.32 to 1.98)	0.33
Malay	1.95 (1.28 to 2.63)	2.24 (-1.76 to 6.24)	0.66	1.46 (0.95 to 1.97)	1.49 (-2.33 to 5.31)	0.36	2.04 (1.44 to 2.65)	1.66 (-1.84 to 5.15)	0.87	0.58 (0.18 to 0.99)	0.16 (-2.32 to 2.65)	0.16
Indian	1.52 (0.54 to 2.51)	2.26 (-1.13 to 5.66)	0.39	0.83 (0.09 to 1.56)	2.06 (-0.98 to 5.10)	0.13	1.12 (0.33 to 1.92)	2.54 (-1.17 to 6.26)	0.09	0.30 (-0.32 to 0.92)	0.48 (-1.66 to 2.62)	0.63
P[d]	0.76	0.95		0.14	0.68		0.28	0.51		0.21	0.19	

BMI, body mass index; GDM, gestational diabetes mellitus; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure

[a] A total of 61 women were excluded from the analysis because of missing information on gestational diabetes status. The results were derived from multiple linear regression models with adjustment for maternal age, education level, parity status, smoking history, body height and heart rate.

[b] for interaction between GDM and BMI

[c] for interaction between ethnicity and BMI

[d] for interaction between ethnicity and skinfold thickness

3.4 Discussion

In a large cohort of south Asian women of Chinese, Malay and Indian descent, we found positive associations between 2nd trimester adiposity (measured by BMI and sum of skinfold thickness) and peripheral and central blood pressures. The associations were generally stronger in Chinese women and women with gestational diabetes. Particularly, greater adiposity was associated with higher blood pressures among Chinese women with gestational diabetes.

Our study is one of the few that has evaluated adiposity in relation to both peripheral and central blood pressures. The positive association is consistent with previous reports suggesting that obesity is a potential modifiable risk factor for elevated blood pressures in pregnancy[30, 88-90, 93] and pregnancy hypertensive disorders (summarized in O'Brien *et al*[77]). For example, in a longitudinal cohort study of 6902 pregnant women in the Netherlands, a one unit increase in pre-pregnancy BMI was associated with 0.89 and 0.74 mmHg higher SBP and DBP, respectively, at 30 weeks gestation[88]; our study estimates were similar at 1.19 and 0.74 mmHg for the corresponding blood pressures although both BMI and blood pressures were measured at the 2nd trimester. Our findings of positive associations between maternal adiposity and central blood pressures are consistent with studies examining arterial stiffness and adiposity in adult population[106-108] as well as in an earlier report by Fujime *et al.* [30] on 830 healthy Japanese women (crude Pearson's correlation between pre-pregnancy BMI with CASP 0.31). Studies have suggested that central blood pressures may better reflect arterial stiffness and provide incremental value above and beyond brachial blood pressures.[63, 109] Thus, more investigations are needed to evaluate whether obesity is related to

central blood pressures and whether weight loss is linked to improvement in central blood pressures.

To our knowledge, this is the first study to report the ethnic differences in the association between adiposity and blood pressures during pregnancy in Asian populations. Malay pregnant women had the highest prevalence of obesity, but the adiposity-blood pressure association was strongest in Chinese compared to Malay or Indian women. The ethnic disparity of the relation between maternal adiposity and blood pressures in our study is consistent with previous studies that examined this relationship in non-pregnant populations,[83, 110-113] particularly, some studies have shown that the adiposity-blood pressure association is stronger in Chinese than non-Chinese Asians.[83, 113] For example, Razak *et al.* [83] found that Chinese men and women had the highest blood pressures at the same BMI compared to South Asians and Europeans in 1078 non-pregnant adults in Canada. Similar results have been reported in another study in Canada, where Foulds *et al.* [113] found that East Asian women had significantly higher risk of hypertension than South Asian and European women at the same BMI level.

The exact reasons for the ethnic difference in the association are unclear. But our findings are supported by a recent study where Khoo *et al.* [100] enrolled 4804 Chinese, Malay, and Asian-Indian residents of Singapore and found that the increases in insulin resistance and C-reactive protein for each unit increase of BMI were greater in Chinese than that in Malay or Asian-Indian adults. Insulin resistance and inflammation have been implicated as potential mechanisms for high blood pressures: obesity may induce endothelial cell chronic inflammatory and oxidative stress, then cause insulin resistance [114]

and eventually lead to impaired local and systemic vascular functions.[76] As postulated by Khoo *et al.* [100], "Chinese may have a lower capacity for fat storage and an excess caloric intake may results in greater metabolic perturbations". Genetic differences and various patterns of immigration and lifestyle between the ethnic groups may also play a role. However, we did not measure insulin resistance or C-reactive protein in our study population and we did not have information on immigration, lifestyle or genetic variations; thus, we are unable to directly test whether these mechanisms explains the ethnic disparity. Further investigations are strongly needed to explore the potential reasons.

The greater associations between maternal adiposity and blood pressures observed in women with gestational diabetes suggest that the presence of gestational diabetes and high maternal BMI may have synergistic effects on arterial compliance in pregnancy as both are independent risk factors for hypertensive complications in pregnancy.[87] Women with gestational diabetes have raised inflammatory markers and insulin resistance in early pregnancy or even before pregnancy,[115, 116] and therefore, we speculate that obese women with gestational diabetes may have greater inflammation and insulin resistance than those with either condition or in isolation, leading to higher blood pressures. We further found that the interaction between obesity and gestational diabetes was only significant in Chinese women. There is some preliminary evidence to suggest ethnic differences in obesity related insulin resistance[117] and gestational diabetes in pregnancy[118, 119] and in adult population.[100] For example, in a previous report by Retnakaran *et al.* [117], the correlation between maternal adiposity during pregnancy and insulin

resistance is greatest in Asian women and less apparent in South Asian women. Therefore, it is possible that the synergistic effect between obesity and gestational diabetes on blood pressures is greater in certain ethnic group.

Our study is the first to examine interactions between maternal adiposity, ethnicity and gestational diabetes in relation to blood pressures in Asian women. We included both BMI and sum of skinfold thickness as adiposity measures, and the generally consistent results are reassuring. Standard operating procedures for measuring maternal anthropometry and blood pressures reduced the measurement errors in the exposures and outcomes. One strength of our study is that we included not only peripheral blood pressures but also central blood pressures to assess haemodynamic adaptation.

This study has several limitations. First, due to the cross-sectional nature, we cannot establish temporality for the relationship between adiposity and blood pressures in the 2nd trimester. Future studies should incorporate multiple measurements on adiposity and blood pressures before and during pregnancy to capture trimester-specific temporal changes. Second, because we did not measure the adiposity before pregnancy, we could not evaluate the association between pre-pregnancy adiposity and weight gain during pregnancy with blood pressure outcomes. The 2nd trimester BMI and skinfold thicknesses may not accurately reflect maternal adiposity due to the growing fetus and fluid accumulation; therefore, measurement error and misclassification is possible. Third, despite studying a large cohort of Asian women, we may still be under-powered due to the smaller numbers of Malays and Indians. Lastly, residual confounding cannot be fully ruled out; for example, we did not have information on diet, physical activity and medication use.

In conclusion, our study provides supportive evidence that maternal adiposity is associated with higher central and peripheral blood pressures during pregnancy. The novel finding that the association varies by ethnicity and gestational diabetes requires further confirmation. Our observation that obesity has the strongest impact on blood pressures in Chinese women with gestational diabetes has substantial clinical and public health significance, suggesting that this group of women may need special attention on weight management. Our results also point to the need for urgent translational research to investigate whether weight reduction in obese pregnant women could have beneficial effects on blood pressures.

Supplemental Table 3-1. Maternal Characteristics by Study Inclusion to the Present Study

Maternal Characteristics	n	Included in study	n	Excluded from study	*P**
Age (years)	799	30.4 ± 5.2	342	30.1 ± 5.3	0.27
Ethnicity					0.25
Chinese	435	54.4%	186	54.4%	
Malay	229	28.7%	86	25.2%	
Indian	135	16.9%	70	20.5%	
Education					0.81
Primary to secondary	256	32.4%	110	31.9%	
GCE/Vocational/Polytechnic	276	34.9%	127	36.9%	
Tertiary	257	32.6%	107	31.1%	
Parity					0.99
Nulliparous	329	42.9%	129	43.3%	
Multiparous	438	57.1%	169	56.7%	
Smoking history					0.36
Never smokers	673	86.7%	235	84.5%	
Ever smokers	103	13.3%	43	15.5%	
Pre-existing chronic hypertension					0.75
No	773	98.0%	338	98.3%	
Yes	16	2.0%	6	1.7%	
Gestational diabetes					0.25
No	602	81.6%	242	84.6%	
Yes	136	18.4%	44	15.4%	
Second trimester BMI (kg/m^2)	799	26.2 ± 4.4	280	26.0 ± 4.5	0.47
Sum of skinfold thickness (mm)	797	79.9 ± 19.6	294	79.8 ± 19.4	0.91
Peripheral BP (mmHg)					
SBP	799	109.5 ± 11.2	30	109.9 ± 9.9	0.85
DBP	799	66.8 ± 8.4	30	65.5 ± 8.9	0.40
Central BP (mmHg)					
SBP	799	96.9 ± 10.1	30	96.9 ± 9.9	0.99
PP	799	30.0 ± 16.3	30	31.4 ± 7.0	0.27
Pulse rate (beats/minute)	799	104.0 ± 16.3	30	106.1 ± 16.9	0.49

BMI, body mass index; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure

Data are mean ± standard deviation or % as specified.

*Using Student *t* test for continuous variables or χ^2 test for categorical variables.

Missing values for variables:; Education = 10; Parity = 32; Smoking history = 23; Gestational diabetes mellitus = 61 and Sum of skinfold thicknesses = 2.

Supplemental Table 3-2. Associations between 2nd Trimester BMI Categories (WHO International and Asian Classifications) and Blood Pressures[a]

		Peripheral Blood Pressures (mmHg)				Central Blood Pressures (mmHg)			
		SBP		DBP		SBP		PP	
	N	ß (95% CI)	*P*	ß (95% CI)	*P*	ß (95% CI)	*P*	ß (95% CI)	*P*
2nd Trimester BMI (kg/m^2)	799	1.19 (1.03 to 1.36)	<0.001	0.76 (0.63 to 0.89)	<0.001	1.02 (0.87 to 1.17)	<0.001	0.26 (0.16 to 0.37)	<0.001
2nd Trimester BMI (International classification)[b]									
Normal weight (≤24.9 kg/m^2)	368	Reference		Reference		Reference		Reference	
Overweight (25.0-29.9 kg/m^2)	279	6.18 (4.60 to 7.76)	<0.001	3.12 (1.91 to 4.33)	<0.001	5.25 (3.79 to 6.71)	<0.001	2.13 (1.14 to 3.12)	<0.001
Obese (≥30.0 kg/m^2)	152	12.95 (10.94 to 14.95)	<0.001	8.58 (7.04 to 10.11)	<0.001	11.18 (9.33 to 13.02)	<0.001	2.59 (1.34 to 3.86)	<0.001
2nd Trimester BMI (Asian classification)[c]									
Normal weight (≤22.9 kg/m^2)	193	Reference		Reference		Reference		Reference	
Overweight (23.0-27.4 kg/m^2)	347	4.75 (2.97 to 6.53)	<0.001	2.50 (1.12 to 3.88)	<0.001	4.19 (2.55 to 5.84)	<0.001	1.70 (0.58 to 2.81)	0.003
Obesity (≥27.5 kg/m^2)	259	12.51 (10.54 to 14.49)	<0.001	7.29 (5.75 to 8.82)	<0.001	10.68 (8.85 to 12.50)	<0.001	3.39 (2.16 to 4.63)	<0.001

BMI, body mass index; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure

[a] Results were derived from multiple linear regression models with adjustment for maternal age, ethnicity, education level, parity status, smoking history, gestational diabetes, body height and heart rate.

[b] BMI categorized according to WHO international classification.

[c] BMI categorized according to WHO classification for Asian population in relation to risk to type 2 diabetes and cardiovascular disease.

CHAPTER 4

POLYUNSATURATED FATTY ACIDS AND BLOOD PRESSURES DURING PREGNANCY IN SOUTH-EAST ASIAN WOMEN

This chapter was based on the paper published in Medicine:

Wai-Yee Lim, Mary Chong, Philip C Calder, Kenneth Kwek, Yap-Seng Chong, Peter D Gluckman, Keith M Godfrey, Seang-Mei Saw and An Pan. Relations of plasma polyunsaturated fatty acids with blood pressures during the 26th and 28th week of gestation in women of Chinese, Malay, and Indian ethnicity. Medicine. 2015; 94(9):e571. doi: 10.1097/MD.0000000000000571.

4.1 Introduction

Hypertensive disorders are major health concerns in pregnancy as they are associated with increased risks of maternal and fetal mortality and morbidity.[20] Therefore, strategies to prevent or limit pregnancy associated hypertension could be very important in reducing maternal and fetal complications. Recent reports on n-3 polyunsaturated fatty acids (PUFAs) supplementation during pregnancy have been shown to prolong pregnancy gestation and increase offspring birth weight,[120, 121] and lower the risk of pregnancy complications,[4,5] although the precise mechanisms are unclear.

Two meta-analyses of randomised trials have suggested that increasing dietary intake of long chain n-3 PUFAs lowers blood pressures, with stronger effects in hypertensive patients.[79, 122] This hypotensive effect of n-3 PUFAs is likely due to several mechanisms including reduced inflammation, improved vascular endothelial function and increased nitric oxide production, effects that are well demonstrated in non-pregnant adult populations.[81, 123] However it is unclear whether the effects persist on maternal blood pressures and pregnancy associated hypertension are less clear,[120, 124-127] and only two trials have examined the effect of n-3 supplementation on maternal blood pressures (Table 4-1).[128, 129] A number of observational studies have evaluated the relations of n-3 PUFAs and pregnancy associated hypertension, with some reporting inverse association,[130-132] while others reporting null[133, 134] or positive relations (Table 4-2).[135-137] The results for n-6 PUFAs were also inconsistent.[130-132, 134, 135, 137] Moreover, no observational study has examined the relations of n-3 and n-6 PUFAs with the continuous measures of maternal blood pressures during pregnancy, particularly in Asian women. Therefore, in

this study we aimed to evaluate the relations of maternal plasma concentrations of n-3 and n-6 PUFAs between 26 - 28 weeks gestation with maternal blood pressures and pregnancy associated hypertension in a birth cohort of Chinese, Malay and Indian women.

Table 4-1: Evidence Table for the Association between n-3 and n-6 PUFAs and Blood pressure Outcomes in Pregnancy (Randomised Controlled Trials)

ID	Year/author	Design	Participants n/country/year enrolled	Exposure(s)	Outcome(s)	Findings
1	2006/Barden et al	RCT (Fish oil vs none)	Women with allergic disease 98/Australia/ (40 treated vs 43 control)	4 capsules/day – fish oil (27.7%EPA + 56% DHA) Olive oil (67% oleic acid) Treated began at 20 weeks gestation	SBP DBP	No significant difference between SBP and DBP between groups.
2	2000/Olsen et al	RCT (fish oil vs olive oil)	Women with previous preterm delivery, hypertension in pregnancy and growth restricted offspring 4 prophylactic trials (232;280; 386; 579) + 2 therapeutic trials (79 – preeclampsia; 63 – growth restriction)/ 19 centres in Denmark, Scotland, Sweden, England, Italy, The Netherlands, Norway, Belgium and Russia/ (?1991 to 1996)	Prevention trial: 4 capsules/day - fish oil (32% EPA + 23% DHA) vs olive oil (oleic acid 72% + linoleic acid 12%) Treated began about 20 weeks gestation Therapeutic trial: 9 capsules/day Treated began at 33 weeks gestation	Recurrence of Gestational hypertension	OR 0.98 (95%CI 0.63 to 1.53)

3	1996/Salvig et al	RCT (Fish oil vs olive oil vs none)	533/ Denmark/(1989-1990) 266 treated with fish oil:136 treated with olive oil:131 control	4 capsules/day - fish oil (32% EPA + 23% DHA) vs olive oil (oleic acid 72% + linoleic acid 12%) Treated began at 30 weeks gestation	SBP DBP	No significant difference between SBP and DBP between groups; Systolic Hypertension:105 vs 53 vs 42 Diastolic Hypertension:14 vs 14 vs 5
4	1995/ Onwude et al	RCT (Fish oil vs placebo-air filled capsules)	233/UK/(1990-1992) (113 treated vs 119 control)	9 capsules - 2.7g Max EPA (Each capsule - EPA 180 mg + DHA 120 mg) Treated at 18-32 weeks gestation	Preeclampsia Gestational hypertension	0.88 (95%CI 0.47 to 1.66) 1.44 (95%CI 0.87 to 1.67)
5	1994/ Blustra-Ramakers et al	RCT (EPA vs placebo-coconut oil)	Women with history of growth restricted offspring 63/The Netherlands/ (1987-1990) (32 treated vs 31control)	12 capsules – 3g EPA Each capsule contained EPA+DPA; provided 0.25 mg EPA Treated began 12-14 weeks gestation	Recurrent Gestational hypertension	EPA: 12/24 (50%) Placebo: 5/15 (33%)
6	1990/Olsen & Secher (Main report 1946)	RCT (fish oil vs control-none)	5644/UK/(1938-1939) (2510 treated vs 2512 control) (The People's League of Health)	EPA and DHA - 0.1g/d Mean length of treated 20 weeks gestation (<15 to >24 weeks)	Preeclampsia Gestational hypertension	OR 0.69 (95%CI 0.53 to 0.89; p=0.0047) 0.95 (95%CI 0.83 to 1.09; p=0.43)

RCT, randomised controlled trial; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SBP, systolic blood pressure; DBP, diastolic blood pressure

Table 4-2: Evidence Table for the Association between n-3 and n-6 PUFAs and Blood pressure Outcomes in Pregnancy (Observational Studies)

ID	Year/author	Design	Participants n/country/ year enrolled	Exposure(s)	Outcome(s)	Findings
1	2007/ Oken et al	Prospective cohort	1718/US/ (1999-2002)	Dietary PUFAs (FFQ) at first trimester n-3 PUFAs – ALA; EPA+DHA n-6 PUFAs – LA; AA EPA+DHA/AA	Preeclampsia; Gestational Hypertension	EPA+DHA: Preeclampsia :OR 0.84 (95%CI 0.69 to 1.03) Gestational Hypertension: OR 1.01 (95%CI 0.95 to 1.08) EPA+DHA/AA: Preeclampsia: OR 0.82 (95%CI 0.89 to 1.01) Gestational Hypertension: OR 0.99 (95%CI 0.93 to 1.07) n-3, n-6, n-3/n-6 not associated with Preeclampsia and Gestational Hypertension
2	2005/ Olafsdottir et al	Prospective cohort	488/Iceland/ (1999-2001)	Fish oil supplementation (Cod liver oil) between 11-15 weeks gestation	Preeclampsia and Gestational Hypertension	All: OR 4.7 (95%CI 1.8 to 12.6) Preeclampsia: OR 4.2 (95%CI 0.8 to 20.9) Gestational Hypertension: OR 5.2 (95%CI 1.5 to 17.8)

3	2001/ Clausen et al	Prospective cohort	3133/Norway/ (1994-1996)	Dietary n-3 and n-6 PUFAs at 17-19 weeks gestation	Preeclampsia	3^{rd} vs 1^{st} tertiles of PUFA: OR 2.6 (95%CI 1.4 to 6.1)
4	2010/ Bakheit et al	Case control (matched for age, parity & gestation)	65 cases: 60 controls/ Sudan/ 2008	Erythrocyte n-3 and n-6 long chain PUFAs at 3^{rd} trimester	Preeclampsia	Cases vs controls for phosphatidylcholine PUFA (% total FA): EPA: 0.09 vs 0.07 ($p<0.001$) DHA: 1.37 vs 1.23 ($p=0.05$) AA: 8.18 vs 808 ($p=0.67$)
5	2007/ Mahomed et al	Case control	170 cases:185 controls/ Zimbabwe/ (1995-1996)	Erythrocyte n-3 and n-6 long chain PUFAs at 12-72 hours postnatally	Preeclampsia	1^{st} vs 4^{th} quartiles of PUFA: n-3: OR 0.42 (95%CI 0.20 to 0.87) n-6: OR 0.85 (95%CI 0.45 to 1.61) n-3/n-6: OR 0.96 (95%CI 0.45 to 2.05)
6	2006/Qiu et al	Case control	100 cases: 100 controls/Peru/ (1997-1998)	Erythrocyte n-3 and n-6 long chain PUFAs non-fasting and pre-labor specimens	Preeclampsia	1^{st} vs 4^{th} quartiles of PUFA: n-3: OR 3.3 (95%CI 1.2 to 9.5) n-6: OR 1.5 (95%CI 0.6 to 3.8)
7	1997/ Kesmodel et al	Nested case control study	Cases 123 (GH)+33 (PE): 184 and 189 controls/ Denmark/	Dietary n-3 PUFAs collected 6 months to 3.5 years after birth	Preeclampsia and Gestational Hypertension	3^{rd} vs 1^{st} categories of fish intake (for n-3 PUFAs): Preeclampsia: OR 0.51 (95%CI 0.12 to 2.23)

			(1989-1991			Gestational Hypertension: OR 0.79 (95%CI 0.27 to 2.34)
8	1995/ Williams et al	Case control	22 cases:40 controls/US/ 1993	Erythrocyte n-3 and n-6 long chain PUFAs collected first postnatal day:	Preeclampsia	1^{st} vs 3^{rd} tertiles of PUFA: n-3: OR 7.63 (95%CI 1.43 to 40.63) n-6: OR 0.10 (95%CI 0.01 to 0.97)

PUFA, polyunsaturated fatty acids; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; AA, arachidonic acid; OR, odds ratio

4.2 Methods

Study Participants

A birth cohort study on Asian women, known as the **G**rowing **U**p in **S**ingapore **T**owards healthy **O**utcomes (GUSTO) study, was initiated to recruit women in their early pregnancy from two public tertiary hospitals with maternity care in Singapore.[85] From 2009 to 2012, the GUSTO study enrolled 1162 Singapore citizens or residents with homogenous parental ethnic Chinese, Malay or Indian background. Women were excluded if they received chemotherapy, psychotropic drugs or had type 1 diabetes. The study was approved by the SingHealth Centralised Institutional Review Board and National Healthcare Group Domain Specific Review Board. Written informed consent was obtained from each study participant.

In the present analysis, we utilized information on maternal plasma phosphatidylcholine (PC) PUFAs and blood pressures measured during the GUSTO antenatal study visit between 26 - 28 weeks gestation. Women with incomplete information on plasma PC PUFAs (n=166) or blood pressures (n=245) were excluded, leaving a final sample of 751 (64.6% of the cohort) women for analysis. Women who were included and excluded from the study had similar ages, education levels and body mass index (BMI), but the excluded women were more likely to be smokers and alcohol drinkers before or during pregnancy than women who were included (17.2% versus 12.7%; 40.2% versus 33.5%, respectively; Supplemental Table 4-1).

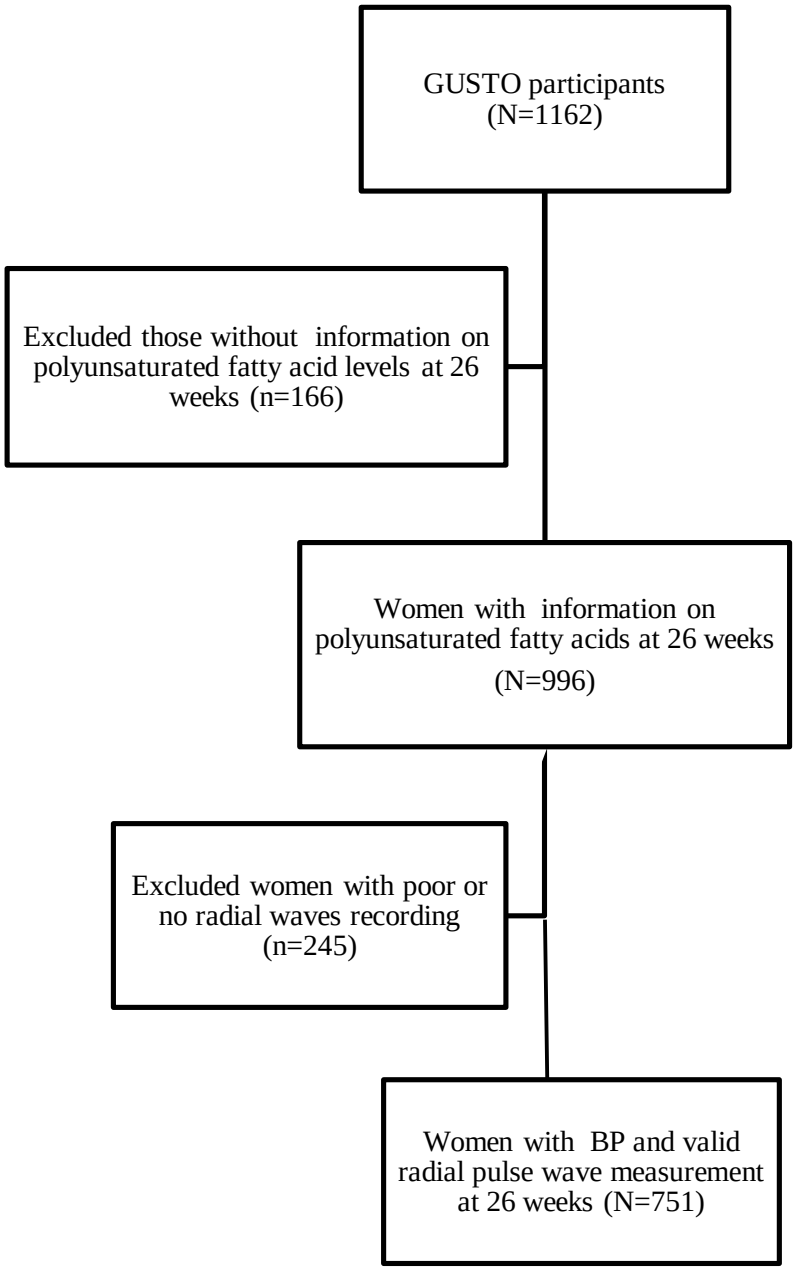


Figure 4-1. Flow Chart of the GUSTO Study Sample Selected for Analysis

Blood Pressure Measurements

Maternal blood pressures and heart rates were taken by trained research coordinators based on a standardized protocol. Peripheral systolic (SBP) and diastolic (DBP) pressures were measured thrice from the brachial arm at 30-60 second intervals with an oscillometric device MC3100 (HealthSTATS International Pte Ltd, Singapore). An average of the three readings was calculated if the difference between readings was less than 10 mmHg; otherwise, measurements were repeated. An A-pulse tonometer (BPro®, HealthSTATS International Pte Ltd, Singapore) was applied on the radial artery of the same arm for continuous sampling of radial artery pressure waveforms over 1 minute, and these waveforms were calibrated using the average of brachial SBP and DBP, respectively. Maternal heart rate and central SBP was then estimated from the calibrated radial artery pressure waveforms using the N-point moving average.[12] Central pulse pressure was calculated as the difference between central SBP and peripheral DBP.

Pregnancy Associated Hypertension

Information on pregnancy associated hypertension, including gestational hypertension and preeclampsia, was ascertained from medical records. The abstracted information was cross-checked by another obstetrician who was involved with the study. In practice, the diagnosis of gestational hypertension included de novo hypertension (defined as peripheral SBP ≥140 mmHg or DBP ≥90 mmHg) without proteinuria after the 20th week of gestation, measured at two separate occasions with at least 4 hours apart.[138] The definition for preeclampsia was hypertension with proteinuria after the 20th

week of gestation. As preeclampsia is a multi-organ disorder, and it may also include impairments in kidney, liver functions and low platelets.[138] As there were only 16 incident cases of gestational hypertension and 12 incident cases of preeclampsia, they were collectively analysed as pregnancy associated hypertension.

Plasma Phosphatidylcholine Fatty Acid Composition

Fasting blood samples were taken between 26 - 28 weeks gestation. Plasma was prepared by centrifugation and was stored at -80°C until analysis. Total lipid extraction was carried out with chloroform/methanol (2:1 v/v) and PC, which contributes about 75% of plasma phospholipids, was isolated by solid phase extraction on aminopropylsilica catridges and eluted with chloroform/methanol (3:2 v/v). Fatty acid methyl esters were generated by reaction of purified PC with 2% sulfuric acid (v/v) at 50°C for 2 hours, extracted into hexane and separated by gas chromatography. A BPX-70 column (30 m × 220 μm; film thickness 0.25 μm) fitted to a Hewlett-Packard HP6890 gas chromatograph was used for separation with helium as the running gas and detection of fatty acid methyl esters by flame ionisation before quantification using the ChemStation software in absolute concentration (μg/mL plasma). Plasma PC fatty acids were expressed as percentages of total plasma PC fatty acids, and the ratio of total n-3 to n-6 PUFAs were calculated accordingly.

Covariates

Information on maternal age, ethnicity and education level, smoking status, alcohol intake before and during pregnancy, hypertension before pregnancy,

physical exercise and dietary supplements during pregnancy were obtained via standardized questionnaires. Maternal anthropometry (height and weight) was measured by trained investigators between 26 - 28 weeks gestation, and BMI was calculated as weight (kg) divided by the square of height (m^2). Women with BMI <25.0 kg/m^2 were categorized as normal weight, 25.0-29.9 kg/m^2 as overweight and ≥30.0 kg/m^2 as obese.[105] Oral glucose tolerance test was performed and gestational diabetes was defined as fasting glucose ≥7.0 mmol/L or 2-hour glucose ≥11.1 mmol/L.

Statistical Analysis

Crude trends of maternal baseline characteristics and pregnancy outcomes across tertiles of n-3 and n-6 PUFAs were done using Mantel-Haenszel test. The associations between plasma PC fatty acids and continuous measures of maternal peripheral and central blood pressures were examined using multiple linear regression analysis. The relation of fatty acids with pregnancy associated hypertension was examined using multiple logistic regression with exclusion of women with hypertension before pregnancy. All analyses were adjusted for maternal age, ethnicity, education level, exercise, smoking status and alcohol intake before or during pregnancy, BMI and height between 26 - 28 weeks gestation, gestational diabetes, heart rate and the use of fish oil supplements. As there were only 14 women with hypertension before pregnancy, we did not adjust for this covariate in our main analysis. However, to test the robustness of our results, we adjusted for maternal hypertension before pregnancy or excluded them in our sensitivity analysis, and the results remained unchanged. We further adjusted for plasma monounsaturated fatty acids (MUFAs) in the sensitivity analysis. We also repeated our analysis for

the continuous measures of blood pressure outcomes in a subgroup of 383 women who did not use fish oil supplements.

Effect modification by maternal ethnicity was performed for the continuous measures of maternal blood pressures, but not for the binary outcomes of pregnancy associated hypertension due to the limited number of cases (n=28). To assess for ethnic modification, a multiplicative interaction term between ethnicity (Chinese, Malay, Indian) and plasma PC fatty acids (continuous variable) was added in the models. Ethnicity stratified analysis was performed and the likelihood ratio test was used to examine the interaction effects. Stata version 11.2 (Statacorp, College Station, Texas) was used for analysis, and two-tailed P value <0.05 was considered statistically significant.

4.3 Results

The women included in our analysis were predominantly Chinese (53.8%), followed by Malay (28.8%) and Indians (17.3%). Those with higher plasma PC n-3 PUFAs tended to have lower BMIs and were more physically active compared to those with higher plasma PC n-6 PUFAs (Table 4-2). The crude incidence of pregnancy associated hypertension was 3.9% (n=28) among women who were free from hypertension before pregnancy. Plasma PC n-3 PUFAs were inversely correlated with gestational hypertension or preeclampsia (*P* for trend = 0.02), but for plasma PC n-6 PUFAs, a marginal trend of positive correlation was observed instead (*P* for trend = 0.05)

PUFAs accounted for about 40% of plasma PC fatty acids, with 6.4% as total n-3 PUFAs and 34.2% as total n-6 PUFAs (Table 4-3). The n-3 PUFAs identified were α-linolenic acid (ALA, 18:3n-3; 0.2%), long chain n-3 [eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3) and docosahexanoic acid (22:6n-3); 5.9%], whereas the n-6 PUFAs included linoleic acid (18:2n-6; 21.7%), dihomo-γ-linolenic acid (20:3n-6; 3.9%) and arachidonic acid (20:4n-6; 7.9%). Chinese women had the highest total n-3 PUFAs (6.7%), whereas, Indian women had the highest total n-6 PUFAs (35.2%). Furthermore, women who took fish oil supplements tended to have higher total n-3 PUFAs (6.8% versus 6.0%), higher long chain n-3 PUFAs (6.4% versus 5.7%) and lower n-6 PUFAs (33.9% versus 34.4%) compared to women who did not take fish oil supplements (Supplemental Table 4-2).

Table 4-3. Characteristics of Women by Tertiles of Plasma PC n-3 and n-6 Polyunsaturated Fatty Acids (PUFAs) at 26 - 28 Weeks Gestation

		n-3 PUFAs			*P*	n-6 PUFAs			*P*
	n	**1st Tertile**	**2nd Tertile**	**3rd Tertile**	**(Trend)**	**1st Tertile**	**2nd Tertile**	**3rd Tertile**	**(Trend)**
Age (year)	751	29.9 ± 5.2	30.0 ± 5.2	31.7 ± 4.8	<0.001	31.1 ± 5.1	30.4 ± 5.1	30.1 ± 5.2	0.04
Ethnicity (%)									
Chinese	404	114 (45.8%)	122 (48.4%)	168 (67.2%)	<0.001	152 (61.8%)	133 (50.2%)	119 (49.6%)	<0.001
Malay	217	82 (32.9%)	82 (32.5%)	53 (21.2%)		64 (26.0%)	88 (33.2%)	65 (27.1%)	
Indian	130	53 (21.3%)	48 (19.0%)	29 (11.6%)		30 (12.2%)	44 (16.6%)	56 (23.3%)	
Education (%)									
Primary to secondary	232	94 (38.4%)	82 (33.1%)	56 (22.5%)	<0.001	71 (29.1%)	86 (32.9%)	75 (31.6%)	0.49
GCE/Vocational/Polytechnic	265	81 (33.1%)	90 (36.3%)	94 (37.8%)		88 (36.1%)	92 (35.2%)	85 (35.9%)	
Tertiary	245	70 (28.6%)	76 (30.6%)	99 (39.8%)		85 (34.8%)	83 (31.8%)	77 (32.5%)	
Exercise (%)									
None to gentle exercise	547	188 (75.8%)	184 (73.0%)	175 (70.3%)	0.16	162 (66.1%)	202 (76.5%)	183 (76.2%)	0.01
Moderate to strenuous exercise	202	60 (24.2%)	68 (26.9%)	74 (29.7%)		83 (33.9%)	62 (23.5%)	57 (23.8%)	
Body Mass Index (kg/m^2)	737	26.7 ±4.5	26.5 ± 4.6	25.5 ± 3.9	0.001	25.6 ± 4.1	26.3 ± 4.5	26.7 ± 4.2	0.02
Height (cm)	744	157.8 ± 5.4	158.5 ±5.7	158.7 ± 5.8	0.03	157.8 ±5.6	158.5 ± 5.3	158.8 ± 6.0	0.14
Smoking status (%)									
Non-smoker	653	212 (85.8%)	206 (82.1%)	235 (94.0%)	0.006	212 (86.2%)	228 (86.7%)	213 (89.1%)	0.33
Ever smoker	95	35 (14.2%)	45 (17.9%)	15 (6.0%)		34 (13.8%)	35 (13.3%)	26 (10.9%)	
Alcohol Intake (%)									
No	486	163 (67.9%)	164 (65.9%)	159 (65.7%)	0.61	141 (58.8%)	179 (70.2%)	166 (70.3%)	0.007
Yes	245	77 (32.1%)	85 (34.1%)	83 (34.3%)		99 (41.2%)	76 (29.8%)	70 (29.7%)	
Gestational Diabetes (%)									
No	577	194 (83.6%)	197 (82.8%)	186 (80.2%)	0.33	196 (82.4%)	207 (85.2%)	174 (78.7%)	0.33
Yes	125	38 (16.4%)	41 (17.2%)	46 (19.8%)		42 (17.6%)	36 (14.8%)	47 (21.3%)	

Hypertension before pregnancy (%)									
No	722	238 (97.5%)	243 (98.4%)	241 (98.4%)	0.50	239 (98.4%)	255 (98.5%)	228 (97.4%)	0.47
Yes	14	6 (2.5%)	4 (1.6%)	4 (1.6%)		4 (1.6%)	4 (1.5%)	6 (2.6%)	
Pregnancy Associated Hypertension * (%)									
Normotensive pregnancy	694	224 (94.1%)	233 (95.9%)	237 (98.4%)		233 (97.5%)	247 (96.9%)	214 (93.9%)	
Gestational hypertension	16	8 (3.4%)	5 (2.1%)	3 (1.2%)	0.02	4 (1.7%)	4 (1.6%)	8 (3.5%)	0.05
Preeclampsia	12	6 (2.5%)	5 (2.1%)	1 (0.4%)		2 (0.8%)	4 (1.6%)	6 (2.6%)	
Fish Oil Supplementation									
No	383	146 (65.5%)	129 (58.4%)	108 (45.9%)	<0.001	111 (49.3%)	130 (55.8%)	142 (64.2%)	0.002
Yes	296	77 (34.5%)	92 (41.6%)	127 (54.0%)		114 (50.7%)	103 (44.2%)	79 (35.8%)	

PC= phosphatidylcholine, PUFAs= polyunsaturated fatty acids.

Data are presented in n (column %) or mean ± SD.

*Incident cases were reported having excluded 14 women with hypertension before pregnancy.

P values were derived from Cochran-Mantel-Haenszel test. Variables with missing information: education (n=9), exercise (n=2), body mass index (n=14), height (n=7), smoking status (n=3), alcohol intake (n=20), gestational diabetes (n=49), hypertension before pregnancy (n=15) and fish oil supplementation (n =72). They were coded as missing.

Table 4-4. Composition of Maternal Plasma PC Fatty Acids (% of Total Fatty Acids) at 26 - 28 Weeks Gestation by Ethnicity

Plasma Fatty Acids	Overall	Chinese	Malay	Indian	*P*
n	751	404	217	130	
SFAs	45.8 ± 3.2	46.0 ± 3.1	45.1 ± 3.1	46.5 ± 3.4	<0.001
MUFAs	13.6 ± 2.3	13.5 ± 2.1	14.6 ± 2.3	12.3 ± 2.0	<0.001
PUFAs	40.5 ± 3.3	40.5 ± 3.4	40.2 ± 3.1	41.1 ± 3.6	0.05
Total n-3 PUFAs	6.3 ± 1.9	6.7 ± 1.9	6.0 ± 1.7	5.9 ± 1.8	<0.001
α-linolenic acid [18:3n-3]	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.12
Long chain n-3 PUFAs	5.9 ± 1.8	6.3 ± 1.9	5.6 ± 1.6	5.6 ± 1.8	<0.001
Total n-6 PUFAs	34.2 ± 3.2	33.8 ± 3.1	34.2 ± 3.0	35.2 ± 3.7	<0.001
Linoleic acid [18:2n-6]	21.7 ± 3.3	21.6 ± 3.2	22.1 ± 3.4	21.2 ± 3.2	0.02
Dihomo-γ-linolenic acid [20:3n-6]	3.9 ± 1.2	3.7 ± 1.2	4.1 ± 1.2	4.4 ± 1.3	<0.001
Arachidonic acid [20:4n-6]	7.9 ± 1.7	7.8 ± 1.5	7.3 ± 1.5	8.9 ± 1.9	<0.001
n-3/n-6 ratio	0.19 ± 0.06	0.20 ± 0.06	0.18 ± 0.06	0.17 ± 0.06	<0.001

PC= phosphatidylcholine, SFAs =saturated fatty acids, MUFAs= monounsaturated fatty acids, PUFAs= polyunsaturated fatty acids.

Data are presented in mean ± SD. P values were derived from one way analysis of variance test.

Relation of Plasma n-3 PUFAs to Maternal Blood Pressures

After multivariate adjustment, higher total and long chain n-3 PUFAs and n-3/n-6 ratio was associated with lower peripheral SBP (Table 4-4 and Figure 4-2): the mean (95% confidence interval [CI]) was -0.51 (-0.89 to -0.13) mmHg for a one-percent increase in total n-3 PUFAs, and -0.52 (-0.92 to -0.13) mmHg for a one-percent increase in long chain n-3 PUFAs, and -1.51 (-2.63 to -0.38) mmHg for a 0.1-unit increase in the n-3/n-6 ratio. Total and long chain n-3 PUFAs and n-3/n-6 ratio were marginally associated with central SBP and PP, but not with DBP. The results were not materially changed in the sensitivity analyses: (1) further adjustment or exclusion of women with hypertension before pregnancy (Supplemental Table 4-3); (2) further adjustment for plasma MUFAs (Supplemental Table 4-3); and (3) in women without fish oil supplementation (Supplemental Table 4-4).

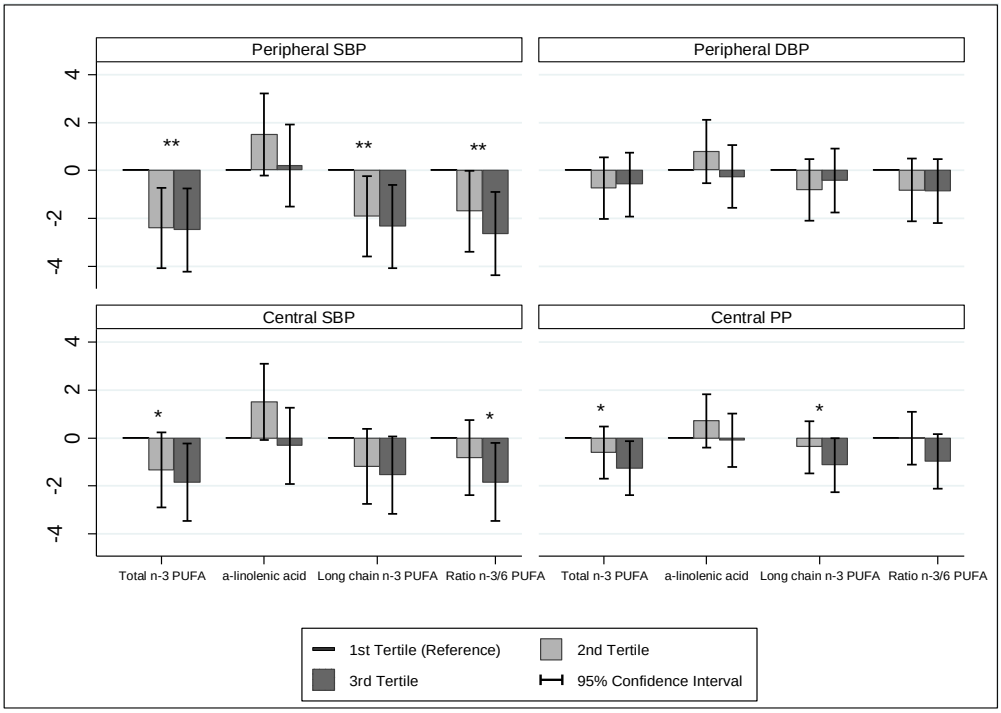


Figure 4-2. Multivariate Adjusted Association between Maternal Plasma PC n-3 PUFAs (Tertiles) and Blood Pressures at 26 - 28 Weeks Gestation

**P* for trend ≤0.05; ***P* for trend <0.001.

Table 4-5. Multivariate Adjusted Association Between Maternal Plasma PC n-3 PUFAs and Blood Pressures at 26 - 28 Weeks Gestation.

	Blood Pressure Outcomes					
	Model 1		Model 2		Model 3	
Fatty Acids (%)	**β (95% CI)**	***P***	**β (95% CI)**	***P***	**β (95% CI)**	***P***
Peripheral SBP						
Total n-3 PUFAs	-0.65 (-1.08 to -0.23)	0.003	-0.48 (-0.86 to -0.11)	0.01	-0.51 (-0.89 to -0.13)	0.008
α-linolenic acid	-2.95 (-8.81 to 2.91)	0.32	-0.02 (-5.18 to 5.14)	0.99	-0.11 (-5.28 to 5.06)	0.97
Long chain n-3 PUFAs	-0.68 (-1.13 to -0.24)	0.003	-0.50 (-0.89 to -0.11)	0.01	-0.52 (-0.92 to -0.13)	0.01
n-3/n-6 ratio*	-2.17 (-3.43 to -0.91)	0.001	-1.44 (-2.55 to -0.33)	0.01	-1.51 (-2.63 to -0.38)	0.009
Peripheral DBP						
Total n-3 PUFAs	-0.23 (-0.56 to 0.09)	0.15	-0.09 (-0.39 to 0.20)	0.52	-0.08 (-0.38 to 0.21)	0.58
α-linolenic acid	-3.43 (-7.83 to 0.97)	0.13	-0.81 (-4.79 to 3.17)	0.69	-1.03 (-5.01 to 2.95)	0.61
Long chain n-3 PUFAs	-0.23 (-0.57 to 0.10)	0.17	-0.09 (-0.39 to 0.21)	0.55	-0.07 (-0.38 to 0.23)	0.63
n-3/n-6 ratio*	-0.83 (-1.78 to 0.12)	0.09	-0.23 (-1.08 to 0.63)	0.60	-0.19 (-1.05 to 0.68)	0.67
Central SBP						
Total n-3 PUFAs	-0.49 (-0.88 to -0.11)	0.01	-0.30 (-0.65 to 0.05)	0.09	-0.31 (-0.67 to 0.04)	0.09
α-linolenic acid	-3.63 (-8.96 to 1.69)	0.18	-1.15 (-5.96 to 3.66)	0.64	-1.33 (-6.15 to 3.48)	0.59
Long chain n-3 PUFAs	-0.50 (-0.90 to -0.09)	0.02	-0.29 (-0.65 to 0.08)	0.12	-0.30 (-0.67 to 0.07)	0.12
n-3/n-6 ratio*	-1.60 (-2.74 to -0.46)	0.01	-0.89 (-1.93 to 0.15)	0.09	-0.91 (-1.96 to 0.13)	0.09
Central PP						
Total n-3 PUFAs	-0.26 (-0.51 to 0.00)	0.05	-0.12 (-0.45 to 0.04)	0.10	-0.13 (-0.48 to 0.02)	0.07
α-linolenic acid	-0.20 (-3.69 to 3.28)	0.91	-0.34 (-3.69 to 3.01)	0.84	-0.30 (-3.66 to 3.06)	0.86
Long chain n-3 PUFAs	-0.26 (-0.52 to 0.00)	0.05	-0.20 (-0.45 to 0.06)	0.13	-0.22 (-0.48 to 0.04)	0.09
n-3/n-6 ratio*	-0.77 (-1.53 to -0.02)	0.04	-0.66 (-1.38 to 0.06)	0.07	-0.73 (-1.46 to 0.00)	0.05

PC= phosphatidylcholine, CI= confidence interval, PUFA= polyunsaturated fatty acids, SBP= systolic blood pressure, DBP= diastolic blood pressure, PP= pulse pressure.

*The values were β (95% CI) for blood pressures in 0.1-unit increase of the n-3/n-6 ratio.

Model 1: adjusted for age and ethnicity using multiple linear regression;
Model 2: adjusted for age, ethnicity, education, exercise, alcohol intake, smoking status, BMI and height at 26 – 28 weeks gestation, gestational diabetes and heart rate;
Model 3: adjusted for variables in Model 2 and fish oil supplementation.

Relation of Plasma n-6 PUFAs to Blood Pressures

The relations of total n-6 PUFAs, linoleic acid and arachidonic acid to blood pressure outcomes were not statistically significant, but dihomo-ɣ-linolenic acid was marginally positively associated with peripheral SBP [0.58 (-0.02 to 1.18); P=0.06] and central SBP [0.52 (-0.04 to 1.07); P=0.07] (Table 4-5 and Figure 4-3). The results were not materially different with further adjustment for maternal hypertension before pregnancy or plasma MUFAs (Supplemental Table 4-5), and in subgroup analysis of women who were not supplemented with fish oil (Supplemental Table 4-6).

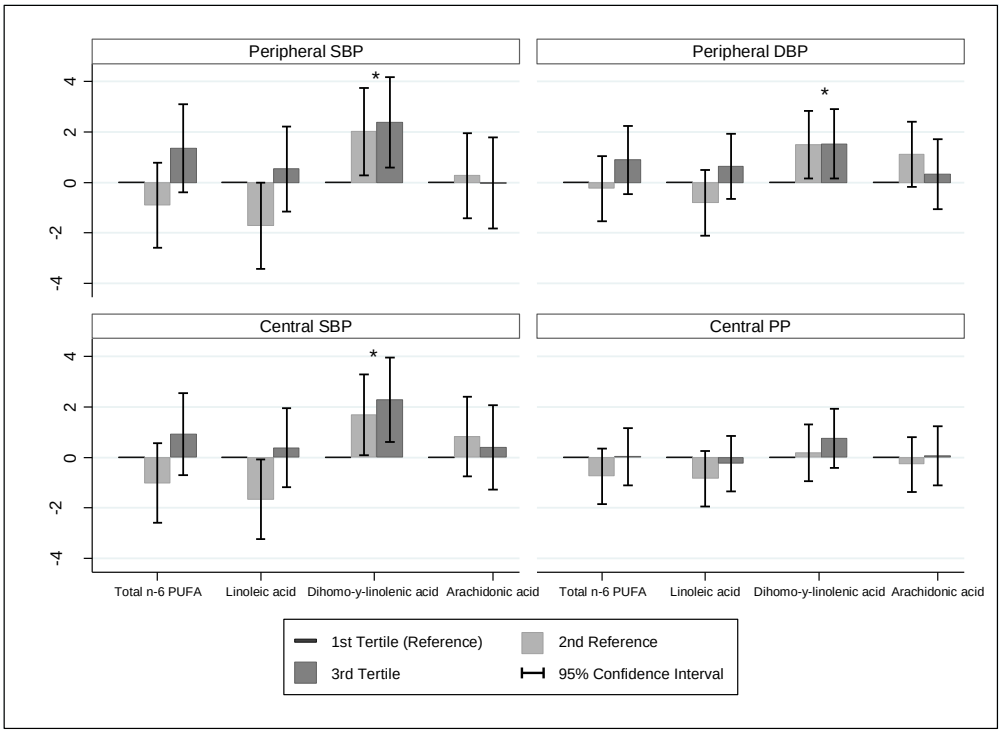


Figure 4-3. Multivariate Adjusted Association between Maternal Plasma PC n-6 PUFAs (Tertiles) and Blood Pressures at 26 - 28 Weeks Gestation

*P for trend ≤0.05

Table 4-6. Multivariate Adjusted Association Between Maternal Plasma PC n-6 PUFAs and Blood Pressures at 26 - 28 Weeks Gestation.

	Blood Pressure Outcomes					
	Model 1		Model 2		Model 3	
Fatty Acids (%)	**β (95% CI)**	***P***	**β (95% CI)**	***P***	**β (95% CI)**	***P***
Peripheral SBP						
Total n-6 PUFAs	0.35 (0.10 to 0.59)	0.006	0.18 (-0.04 to 0.40)	0.11	0.18 (-0.04 to 0.40)	0.11
Linoleic acid	0.03 (-0.21 to 0.27)	0.80	0.14 (-0.07 to 0.35)	0.19	0.14 (-0.07 to 0.35)	0.19
Dihomo-γ-linolenic acid	1.42 (0.76 to 2.08)	<0.01	0.58 (-0.02 to 1.17)	0.06	0.58 (-0.02 to 1.18)	0.06
Arachidonic acid	0.45 (-0.05 to 0.94)	0.08	-0.22 (-0.66 to 0.22)	0.33	-0.22 (-0.66 to 0.23)	0.34
Peripheral DBP						
Total n-6 PUFAs	0.23 (0.05 to 0.42)	0.01	0.09 (-0.08 to 0.25)	0.32	0.08 (-0.09 to 0.25)	0.35
Linoleic acid	-0.03 (-0.22 to 0.15)	0.71	0.02 (-0.15 to 0.18)	0.85	0.02 (-0.14 to 0.18)	0.83
Dihomo-γ-linolenic acid	0.92 (0.42 to 1.41)	<0.001	0.33 (-0.12 to 0.79)	0.15	0.29 (-0.17 to 0.75)	0.21
Arachidonic acid	0.47 (0.10 to 0.84)	0.01	0.00 (-0.34 to 0.34)	0.99	0.00 (-0.34 to 0.34)	0.99
Central SBP						
Total n-6 PUFAs	0.25 (0.02 to 0.47)	0.03	0.13 (-0.07 to 0.34)	0.20	0.13 (-0.07 to 0.33)	0.22
Linoleic acid	-0.08 (-0.30 to 0.14)	0.46	0.05 (-0.15 to 0.24)	0.64	0.05 (-0.15 to 0.24)	0.63
Dihomo-γ-linolenic acid	1.22 (0.62 to 1.81)	<0.001	0.54 (-0.02 to 1.09)	0.06	0.52 (-0.04 to 1.07)	0.07
Arachidonic acid	0.55 (0.10 to 1.00)	0.02	-0.03 (-0.44 to 0.38)	0.89	-0.03 (-0.44 to 0.38)	0.89
Central PP						
Total n-6 PUFAs	0.01 (-0.13 to 0.16)	0.85	0.05 (-0.10 to 0.19)	0.52	0.05 (-0.09 to 0.19)	0.50
Linoleic acid	-0.05 (-0.19 to 0.09)	0.51	0.03 (-0.10 to 0.17)	0.65	0.03 (-0.11 to 0.17)	0.66
Dihomo-γ-linolenic acid	0.30 (-0.10 to 0.69)	0.14	0.20 (-0.18 to 0.59)	0.30	0.22 (-0.16 to 0.61)	0.26
Arachidonic acid	0.08 (-0.22 to 0.37)	0.62	-0.03 (-0.32 to 0.26)	0.84	-0.03 (-0.31 to 0.26)	0.85

PC= phosphatidylcholine, CI= confidence interval, PUFA= polyunsaturated fatty acids, SBP= systolic blood pressure, DBP= diastolic blood pressure, PP= pulse pressure.

Model 1: adjusted for age and ethnicity using multiple linear regression;
Model 2: adjusted for age, ethnicity, education, exercise, alcohol intake, smoking status, BMI and height at 26 - 28 Weeks gestation, gestational diabetes and heart rate;
Model 3: adjusted for variables in Model 2 and fish oil supplementation.

Relations between Plasma PUFAs and Pregnancy Associated Hypertension

The relations of total and long chain n-3 PUFAs to pregnancy associated hypertension were statistically significant (Table 4-6). The adjusted odds ratio (95% CI) for pregnancy associated hypertension from one-percent increase in total n-3 PUFAs was 0.76 (0.60 to 0.97) and 0.77 (0.60 to 0.98) in long chain n-3 PUFAs. No significant associations were found between n-6 PUFAs and pregnancy associated hypertension.

Table 4-7. Multivariate Adjusted Relation of Maternal Plasma PC n-3 and 6 PUFAs at 26 - 28 Weeks Gestation with Pregnancy Associated Hypertension

	Pregnancy Associated Hypertension*					
	Model 1		Model 2		Model 3	
Fatty Acids (%)	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
n-3 PUFAs						
Total n-3 PUFAs	0.69 (0.54 to 0.89)	0.004	0.69 (0.53 to 0.90)	0.007	0.76 (0.60 to 0.97)	0.03
Long chain n-3 PUFAs	0.69 (0.54 to 0.90)	0.006	0.69 (0.53 to 0.92)	0.01	0.77 (0.60 to 0.98)	0.04
n-6 PUFAs						
Total n-6 PUFAs	1.14 (1.01 to 1.29)	0.04	1.10 (0.97 to 1.26)	0.15	1.09 (0.97 to 1.22)	0.16
Linoleic acid	1.05 (0.94 to 1.18)	0.40	1.09 (0.96 to 1.24)	0.17	0.82 (0.56 to 1.19)	0.29
Dihomo-ɣ-linolenic acid	1.09 (0.81 to 1.49)	0.55	0.85 (0.57 to 1.26)	0.42	1.05 (0.82 to 1.34)	0.70
Arachidonic acid	1.23 (1.02 to 1.48)	0.03	1.10 (0.87 to 1.40)	0.42	1.07 (0.95 to 1.22)	0.26

PC= phosphatidylcholine, CI= confidence interval, PUFA= polyunsaturated fatty acids, SBP= systolic blood pressure, DBP= diastolic blood pressure, PP= pulse pressure.

Results for α-linolenic acid and ratio of n-3 to n-6 PUFAs were not reported as their plasma concentrations were very low, leading to unstable estimates and large confidence intervals in the regression analysis.

*Pregnancy associated hypertension (n=28) included gestational hypertension (n=16) and preeclampsia (n=12). A total of 14 women with hypertension before pregnancy were excluded from analysis.

Model 1: adjusted for age and ethnicity using multiple logistic regression;
Model 2: adjusted for age, ethnicity, education, exercise, alcohol intake, smoking status, BMI and height at 26 - 28 weeks gestation, gestational diabetes and heart rate;
Model 3: adjusted for variables in Model 2 and fish oil supplementation.

Relations between Plasma PUFAs and Blood Pressures in Different Ethnic Groups

The relations of total and long chain n-3 PUFAs and n-3/n-6 ratio to peripheral SBP, DBP and central SBP varied across maternal ethnic groups (*P* values for interaction ranged from 0.02 to 0.07). The inverse associations tended to be stronger in Chinese women and weaker in Indian women, but non-significantly positive in Malay women (Supplemental Table 4-7).

The positive relations of linoleic acid with SBP and total n-6 PUFAs with DBP were stronger in Indian women compared to Chinese or Malay women (*P* for interaction = 0.02 and 0.05, respectively; Supplemental Table 4-8). In Indian women, higher tertiles of total n-6 PUFAs and linoleic acid were associated with higher peripheral SBP, DBP and central SBP (*P* for trend ranged from 0.002 to 0.09), and higher tertiles of dihomo-ɣ-linoleic acid with higher peripheral DBP and central SBP (*P* for trend = 0.07).

4.4 Discussion

Plasma PC PUFAs reflect both intake of those fatty acids[139] and levels of those fatty acids in various cells and tissues.[140] Thus, they are good markers of maternal PUFA status. The main findings from this cohort of Asian women are: (1) total and long chain n-3 PUFAs and n-3/n-6 ratio were all inversely associated with peripheral SBP and central SBP and PP between 26 - 28 weeks gestation, whereas dihomo-ɣ-linolenic acid was marginally positively associated with peripheral and central SBP; (2) higher n-3 PUFAs were associated with lower odds of pregnancy associated hypertension; (3) maternal ethnicity modified the relations between plasma PC PUFAs and blood pressures, with stronger inverse associations for n-3 PUFAs in Chinese women, and stronger but positive associations for n-6 PUFAs in Indian women.

Our findings on the inverse relations between plasma PC n-3 PUFAs and maternal blood pressures and pregnancy associated hypertension are consistent with evidence from clinical[78, 123, 141] and epidemiological studies[8,24,25] on the direct and indirect mechanisms of action of n-3 PUFAs[81, 142] and they lend support to the potentially beneficial role of long chain n-3 PUFAs in pregnancy. Although the earlier two trials in pregnant women reported no significant findings,[128, 129] trials in the general population have found consistent hypotensive and cardioprotective effects from n-3 supplementation,[6,7,9] and this has led to the development of several national and international guidelines for their consumption.[81] Further trials on n-3 supplementation are still needed as hypertension in pregnancy is a major complication in pregnancy, and as demonstrated in the general population,[81,

[143] fish oil supplementation may be a useful adjunct to prevent or limit hypertension disorders and associated complications in pregnancy.

This study is one of the few that has comprehensively examined the relations between PUFA status and blood pressures in pregnant Asian women. Our findings are consistent with a recent meta-analysis of 70 randomised clinical trials in non-pregnant populations,[79] that n-3 PUFA provision reduces blood pressures (SBP, -1.52 mmHg; DBP, -0.99 mmHg) compared with placebo. Other studies have found that higher n-3 PUFA concentrations in the blood stream were associated with lower cardiovascular risk.[144] Data from observational studies in non-pregnant populations support the hypotensive effects of n-3 PUFAs measured from dietary intake[145] or plasma phospholipid[145] or serum.[146] For example, plasma phospholipid n-3 PUFAs was inversely associated with hypertension in a cross-sectional study among 1154 Chinese men and women,[145] and serum n-3 PUFAs was inversely associated with peripheral SBP and PP in 778 healthy Finnish men and women.[146]

In contrast to our findings, reports from randomised trials of n-3 PUFA supplementation during pregnancy did not find significant blood pressure reduction[128, 129] or prevention of pregnancy hypertension.[120, 147] This may possibly be due to baseline variation across study populations such as inclusion of women with high risk of pregnancy complications, or differences in the timing and duration of fish oil supplementation. Reports on dietary n-3 PUFAs measured from food frequency questionnaires were inconsistent, with some studies reporting increased risk to preeclampsia from higher dietary n-3 PUFAs intake at mid-trimester[135] or fish oil supplementation at first

trimester.[136] However, other studies reported a lower risk of preeclampsia with higher mean n-3 PUFA intake during the first and second trimesters[130] or null findings with retrospective recall of pregnancy diet.[133] The discrepant findings may be due to the varying gestation period when diet was assessed as well as measurement errors from questionnaires, leading to misclassification of n-3 PUFA status. As for the biomarker assessment of erythrocyte n-3 PUFAs were associated with lower risk to preeclampsia[131, 132] and a lower risk but not statistically significant in another report.[134]

As to the overall positive but not statistically significant associations between plasma PC n-6 PUFAs and maternal blood pressures, the findings are broadly consistent with two observational studies which reported an increased risk of hypertension in pregnancy with higher erythrocyte[134] or plasma n-6 PUFAs.[148] Other reports, however, found null relations with higher dietary[130] or plasma n-6 PUFA levels.[131]

Ethnic differences were found in plasma PC fatty acid composition and in the relations of plasma PC fatty acids to blood pressures. Chinese women had higher plasma PC n-3 PUFAs, possibly because of their higher intake of foods rich in n-3 PUFAs such as eggs, meat (poultry and non-poultry) and fish, whereas the higher plasma PC n-6 PUFAs amongst the Indians may be due to their higher use of n-6 PUFA-rich oils for cooking.[149] We postulate that the greater consumption of dietary n-3 PUFAs among the Chinese women may be linked with lower blood pressures,[145] whereas in Indian women, the high intake of n-6 PUFAs may lead to higher blood pressures.[123, 148] Further investigations into the influences of genetic, dietary and lifestyle factors on n-3 PUFAs and blood pressures in Asian women are needed.

Our study has several strengths. First, as data was acquired from a prospective birth cohort study, we were able to account for important confounders. Second, the blood pressure measurements were performed by trained research personnel, following a standard protocol. Lastly, our study sample of Chinese, Malay and Indian women enabled us to examine the ethnicity related variations in the relations of plasma PC n-3 and 6 PUFAs with blood pressures. However, the results should be interpreted cautiously because of the small sample size in the stratified analysis.

Limitations include the cross sectional nature of our study and therefore we are unable to establish causality of the association between PUFAs and blood pressures. We were also unable to examine maternal blood pressure changes during pregnancy as they were measured between 26 - 28 weeks gestation only. Our findings on the relations of PUFAs with pregnancy associated hypertension were constrained by the lack of study power as there were only 28 cases of pregnancy associated hypertension. Although we have excluded a total of 409 (35.4%) women in the GUSTO study, it is unlikely that selection bias would affect our results as most baseline characteristics between women who were included and excluded in the study were not materially different. Our effect estimates may be affected by residual confounding from imperfectly self-reported measures such as physical activity and supplement use. Lastly, we did not measure dietary intake of PUFAs, and this may have limited the interpretation of our study findings. However, plasma PC n-3 PUFAs have been found to be good markers of n-3 PUFA dietary intake[139, 140] and a dose response relation has been demonstrated recently in a randomized

trial on the dose and time-dependent response of eicosapentaenoic acid and docosahexanoic acid incorporation into various biosamples.[139]

In conclusion, plasma PC n-3 PUFAs were inversely related to peripheral and central SBP and central PP in pregnancy with stronger inverse relations of plasma PC n-3 in Chinese women, but positive relations with plasma PC n-6 PUFAs in Indian women. Higher plasma n-3 PUFAs between 26 - 28 weeks gestation were associated with lower odds of pregnancy associated hypertension.

Supplemental Table 4-1. Characteristics of Women Included and Excluded from the Analysis

	n	Included in Analysis	n	Excluded from Analysis	*P*
Age (year)	751	30.5 ± 5.2	409	29.9 ± 5.3	0.07
Ethnicity (%)					
Chinese	404	53.79%	226	55.26%	0.24
Malay	217	28.89%	101	24.69%	
Indian	130	17.31%	82	20.05%	
Education (%)					
Primary to secondary	232	31.27%	139	34.49%	0.50
GCE/Vocational/Polytechnic	265	35.71%	141	34.99%	
Tertiary	245	33.02%	123	30.52%	
Exercise (%)					
None to gentle exercise	547	73.03%	262	75.29%	0.43
Moderate to strenuous exercise	202	26.97%	86	24.71%	
Body Mass Index (kg/m^2)	737	26.2 ±4.4	343	26.0 ±4.7	0.49
Height (cm)	744	158.4 ±5.6	346	157.9 ±5. 7	0.27
Smoking status (%)					
Non-smoker	653	87.30%	288	82.76%	0.04
Ever smoker	95	12.70%	60	17.24%	
Alcohol Intake (%)					
No	486	66.48%	205	59.77%	0.03
Yes	245	33.52%	138	40.23%	
Gestational Diabetes					
No	577	82.19%	267	82.92%	0.78
Yes	125	17.81%	55	17.08%	
Hypertension before pregnancy (%)					
No	722	98.1%	395	98.5%	0.62
Yes	14	1.9%	6	1.5%	
Pregnancy Associated Hypertension* (%)					
Normotensive Pregnancy	694	96.1%	384	97.2%	0.63
Gestational Hypertension	16	2.2%	6	1.5%	
Preeclampsia	12	1.7%	5	1.3%	
% of total plasma PC fatty acids					
SFAs	751	45.8 ± 3.2	245	45.8 ± 3.9	0.98
MUFAs	751	13.6 ± 2.3	245	13.6 ± 2.3	0.63
PUFAs	751	40.5 ± 3.3	245	40.6 ± 4.2	0.78
Total n-3 PUFAs	751	6.4 ± 1.9	245	6.3 ± 1.8	0.72
α-linolenic acid	751	0.2 ± 0.1	245	0.2 ± 0.2	0.78
Long chain n-3 PUFAs	751	5.9 ± 1.8	245	5.9 ± 1.7	0.74
Total n-6 PUFAs	751	34.2 ± 3.2	245	34.3 ± 4.0	0.63
Linoleic acid	751	21.7 ± 3.3	245	21.9 ± 3.9	0.45
Dihomo-gamma-linolenic acid	751	3.9 ± 1.2	245	3.9 ± 1.4	0.39
Arachidonic acid	751	7.9 ± 1.7	245	7.9 ± 1.7	0.87
n-3/n-6 ratio	751	0.19 ± 0.06	245	0.19 ± 0.05	0.70

PC= phosphatidylcholine, SFAs= saturated fatty acid, MUFAs= monounsaturated fatty acids, PUFAs= polyunsaturated fatty acids.

Data are presented in % or mean ± SD. P values were derived from chi-squared tests for categorical variables, and Student t-tests for continuous variables.

* Incident cases were reported having excluded 14 women with hypertension before pregnancy.

A total of 751 women were included for analysis. Variables with missing information: education (n=9), exercise (n=2), body mass index (n=14), height (n=7), smoking status (n=3), alcohol intake (n=20), gestational diabetes (n=49), hypertension before pregnancy (n=15) and fish oil supplementation (n =72). They were coded as missing.

Supplemental Table 4-2. Composition of Maternal Plasma PC PUFAs (% of Total Fatty Acids) at 26 - 28 Weeks Gestation by Fish Oil Supplementation Status

	Not Supplemented	Supplemented	*P*
n	383	296	
Total n-3 PUFAs	6.06 ± 1.79	6.82 ± 1.91	<0.001
α-linolenic acid [18:3 (n3)]	0.22 ± 0.15	0.21 ± 0.12	0.53
Long chain n-3 PUFAs	5.66 ± 1.73	6.44 ± 1.83	<0.001
Total n-6 PUFAs	34.37 ± 3.35	33.90 ± 3.14	0.07
Linoleic acid [18:2 (n6)]	21.58 ± 3.39	21.77 ± 3.19	0.47
Dihomo-γ-linolenic acid [20:3 (n6)]	4.15 ± 1.22	3.68 ± 1.16	<0.001
Arachidonic acid [20:4 (n6)]	7.94 ± 1.74	7.84 ± 1.63	0.45
n-3/n-6 ratio	0.17 ± 0.06	0.20 ± 0.06	<0.001

PC= Phosphatidylcholine, PUFAs= polyunsaturated fatty acids.

Data are presented in mean ± SD and P values were derived from Student t-tests

Supplemental Table 4-3. Sensitivity Analysis for the Association between Maternal Plasma PC n-3 PUFAs and Blood Pressures at 26 - 28 Weeks Gestation

	Blood Pressure Outcomes							
	Final Model		Final Model Adjusted for Hypertension Before Pregnancy		Final Model (Exclusion of Hypertension Before Pregnancy Cases)		Final Model Adjusted for MUFAs	
Fatty Acids (%)	**β (95% CI)**	***P***	**β (95% CI)**	***P***	**β (95% CI)**	***P***	**β (95% CI)**	***P***
Peripheral SBP								
Total n-3 PUFAs	-0.51 (-0.89 to -0.13)	0.008	-0.51 (-0.89 to -0.13)	0.008	-0.50 (-0.89 to -0.12	0.01	-0.48 (-0.88 to -0.09)	0.02
α-linolenic acid	-0.11 (-5.28 to 5.06)	0.97	-0.11 (-5.28 to 5.05)	0.97	0.24 (-5.00 to 5.49)	0.93	0.27 (-4.94 to 5.48)	0.92
Long chain n-3 PUFAs	-0.52 (-0.92 to -0.13)	0.01	-0.53 (-0.92 to -0.13)	0.009	-0.53 (-0.93 to -0.12)	0.01	-0.50 (-0.91 to -0.08)	0.02
n-3/n-6 ratio*	-1.51 (-2.63 to -0.38)	0.009	-1.52 (-2.64 to -0.39)	0.008	-1.49 (-2.64 to -0.34)	0.01	-1.44 (-2.58 to -0.29)	0.01
Peripheral DBP								
Total n-3 PUFAs	-0.08 (-0.38 to 0.21)	0.58	-0.08 (-0.37 to 0.21)	0.58	-0.09 (-0.39 to 0.20)	0.52	-0.02 (-0.32 to 0.29)	0.91
α-linolenic acid	-1.03 (-5.01 to 2.95)	0.61	-0.99 (-4.95 to 2.97)	0.62	-1.18 (-5.22 to 2.85)	0.56	-0.65 (-4.65 to 3.35)	0.75
Long chain n-3 PUFAs	-0.07 (-0.38 to 0.23)	0.63	-0.07 (-0.38 to 0.23)	0.63	-0.09 (-0.41 to 0.22)	0.55	-0.01 (-0.32 to 0.31)	0.97
n-3/n-6 ratio*	-0.19 (-1.05 to 0.68)	0.67	-0.19 (-1.05 to 0.68)	0.67	-0.23 (-1.11 to 0.66)	0.62	-0.07 (-0.95 to 0.81)	0.88
Central SBP								
Total n-3 PUFAs	-0.31 (-0.67 to 0.04)	0.09	-0.31 (-0.66 to 0.04)	0.09	-0.32 (-0.68 to 0.04)	0.08	-0.28 (-0.65 to 0.10)	0.15
α-linolenic acid	-1.33 (-6.15 to 3.48)	0.59	-1.26 (-6.06 to 3.53)	0.60	-0.97 (-5.85 to 3.92)	0.69	-1.00 (-5.85 to 3.84)	0.68
Long chain n-3 PUFAs	-0.30 (-0.67 to 0.07)	0.12	-0.29 (-0.66 to 0.07)	0.12	-0.32 (-0.69 to 0.06)	0.10	-0.26 (-0.64 to 0.13)	0.19
n-3/n-6 ratio*	-0.91 (-1.96 to 0.13)	0.09	-0.91 (-1.95 to 0.13)	0.09	-0.94 (-2.01 to 1.30)	0.08	-0.83 (-1.89 to 0.23)	0.12
Central PP								
Total n-3 PUFAs	0.13 (-0.48 to 0.02)	0.07	-0.23 (-0.48 to 0.02)	0.07	-0.22 (-0.47 to 0.03)	0.08	0.13 (-0.52 to 0.00)	0.05
α-linolenic acid	-0.30 (-3.66 to 3.06)	0.86	-0.27 (-3.64 to 3.09)	0.87	0.22 (-3.19 to 3.62)	0.90	-0.35 (-3.74 to 3.03)	0.84
Long chain n-3 PUFAs	-0.22 (-0.48 to 0.04)	0.09	-0.22 (-0.48 to 0.04)	0.09	-0.22 (-0.48 to 0.04)	0.09	-0.25 (-0.52 to 0.02)	0.07
n-3/n-6 ratio*	-0.73 (-1.46 to 0.00)	0.05	-0.72 (-1.46 to 0.01)	0.05	-0.71 (-1.46 to 0.03)	0.06	-0.76 (-1.51 to -0.02)	0.04

CI= confidence interval, PC= phosphatidylcholine, PUFAs= polyunsaturated fatty acids, MUFAs= monounsaturated fatty acids, SBP= systolic blood pressure, DBP= diastolic blood pressure, PP= pulse pressure.

*The values were β (95% CI) for blood pressures in 0.1-unit increase of the n-3/n-6 ratio.

There were 14 women with hypertension before pregnancy. The final model was derived from multiple linear regression with adjustment for maternal age, ethnicity education, exercise, alcohol intake, smoking status, BMI and height at 26 - 28 weeks gestation, gestational diabetes, heart rate and fish oil supplementation.

Supplemental Table 4-4. Association Between Maternal Plasma PC n-3 PUFAs and Blood Pressures at 26 - 28 Weeks Gestation in Women without Fish Oil Supplementation

	Fatty Acids (%)							
	1st Tertile	2nd Tertile		3rd Tertile			per 1% Increase	
		β (95% CI)	*P*	β (95% CI)	*P*	*P* (Trend)	β (95% CI)	*P*
Peripheral SBP								
Total n-3 PUFAs	Reference	-0.35 (-2.66 to 1.95)	0.76	-1.88 (-4.35 to 0.59)	0.14	0.19	-0.45 (-0.99 to 0.09)	0.11
α-linolenic acid	Reference	1.59 (-0.84 to 4.03)	0.19	-0.04 (-2.46 to 2.38)	0.97	0.96	-2.08 (-8.57 to 4.40)	0.53
Long chain n-3 PUFAs	Reference	-0.20 (-2.53 to 2.12)	0.86	-1.52 (-3.96 to 0.91)	0.22	0.24	-0.46 (-1.02 to 0.11)	0.12
n-3/n-6 ratio*	Reference	-0.11 (-0.34 to 0.13)	0.36	-0.23 (-0.47 to 0.01)	0.06	0.06	-1.49 (-3.09 to 0.04)	0.07
Peripheral DBP								
Total n-3 PUFAs	Reference	-0.34 (-2.22 to 1.53)	0.72	0.24 (-1.77 to 2.25)	0.81	0.86	0.16 (-0.28 to 0.60)	0.48
α-linolenic acid	Reference	1.13 (-0.84 to 3.11)	0.26	0.00 (-1.96 to 1.97)	0.99	0.99	-2.26 (-7.52 to 3.00)	0.39
Long chain n-3 PUFAs	Reference	-0.34 (-0.22 to 1.55)	0.72	0.88 (-1.10 to 2.86)	0.38	0.43	0.21 (-0.26 to 0.67)	0.38
n-3/n-6 ratio*	Reference	-0.11 (-0.30 to 0.08)	0.26	0.02 (-0.18 to 0.21)	0.86	0.96	0.49 (-0.81 to 1.79)	0.46
Central SBP								
Total n-3 PUFAs	Reference	0.23 (-1.92 to 2.39)	0.83	-1.19 (-3.51 to 1.12)	0.31	0.35	-0.10 (-0.61 to 0.41)	0.69
α-linolenic acid	Reference	1.99 (-0.28 to 4.26)	0.08	-0.32 (-2.58 to 1.93)	0.78	0.75	-2.21 (-8.28 to 3.85)	0.47
Long chain n-3 PUFAs	Reference	0.16 (-2.02 to 2.34)	0.89	-0.40 (-2.68 to 1.88)	0.73	0.75	-0.06 (-0.59 to 0.47)	0.82
n-3/n-6 ratio*	Reference	-0.07 (-0.29 to 0.15)	0.55	-0.13 (-0.35 to 0.09)	0.26	0.26	-0.47 (-1.97 to 1.03)	0.54
Central PP								
Total n-3 PUFAs	Reference	0.58 (-0.89 to 2.06)	0.44	-1.44 (-3.03 to 0.15)	0.08	0.11	-0.26 (-0.62 to 0.09)	0.14
α-linolenic acid	Reference	0.86 (-0.71 to 2.42)	0.28	-0.33 (-1.89 to 1.23)	0.68	0.66	0.05 (-4.14 to 4.23)	0.98
Long chain n-3 PUFAs	Reference	0.49 (-0.99 to 1.99)	0.51	-1.28 (-2.84 to 0.29)	0.11	0.15	-0.27 (-0.64 to 0.09)	0.15
n-3/n-6 ratio*	Reference	0.04 (-1.09 to 1.96)	0.58	-0.14 (-0.29 to 0.01)	0.06	0.09	-0.96 (-1.99 to 0.07)	0.07

CI= confidence interval, PC= phosphatidylcholine, PUFAs= polyunsaturated fatty acids, MUFAs= monounsaturated fatty acids, SBP= systolic blood pressure, DBP= diastolic blood pressure, PP= pulse pressure.

*The values were β (95% CI) for blood pressures in 0.1-unit increase of the n-3/n-6 ratio.

Subgroup analysis was performed in 383 women without fish oil supplementation. Results were derived from multiple linear regression with adjustment for maternal age, ethnicity, education, exercise, alcohol intake, smoking status, BMI and height at 26 - 28 weeks gestation, gestational diabetes and heart rate.

Supplemental Table 4-5. Sensitivity Analysis for the Association between Maternal Plasma PC n-6 PUFAs and Blood Pressures at 26 - 28 Weeks Gestation

	Blood Pressure Outcomes							
	Final Model		Final Model Adjusted for Hypertension Before Pregnancy		Final Model (Exclusion of Hypertension Before Pregnancy Cases)		Final Model Adjusted for MUFAs	
Fatty Acids (%)	**β (95% CI)**	***P***	**β (95% CI)**	***P***	**β (95% CI)**	***P***	**β (95% CI)**	***P***
Peripheral SBP								
Total n-6 PUFAs	0.18 (-0.04 to 0.40)	0.11	0.17 (-0.05 to 0.39)	0.12	0.16 (-0.06 to 0.38)	0.16	0.23 (0.00 to 0.45)	0.05
Linoleic acid	0.14 (-0.07 to 0.35)	0.19	0.13 (-0.08 to 0.34)	0.21	0.13 (-0.08 to 0.34)	0.23	0.19 (-0.03 to 0.42)	0.08
Dihomo-ɣ-linolenic acid	0.58 (-0.02 to 1.18)	0.06	0.57 (-0.02 to 1.17)	0.06	0.57 (-0.04 to 1.18)	0.06	0.52 (-0.11 to 1.14)	0.10
Arachidonic acid	-0.22 (-0.66 to 0.23)	0.34	-0.20 (-0.64 to 0.24)	0.36	-0.24 (-0.68 to 0.21)	0.29	-0.19 (-0.63 to 0.25)	0.40
Peripheral DBP								
Total n-6 PUFAs	0.08 (-0.09 to 0.25)	0.35	0.07 (-0.09 to 0.24)	0.38	0.07 (-0.10 to 0.24)	0.42	0.12 (-0.05 to 0.30)	0.17
Linoleic acid	0.02 (-0.14 to 0.18)	0.83	0.01 (-0.15 to 0.17)	0.42	-0.00 (-0.16 to 0.16)	0.99	0.07 (-0.09 to 0.24)	0.40
Dihomo-ɣ-linolenic acid	0.29 (-0.17 to 0.75)	0.21	0.29 (-0.17 to 0.74)	0.22	0.33 (-0.14 to 0.79)	0.17	0.21 (-0.27 to 0.69)	0.40
Arachidonic acid	0.00 (-0.34 to 0.34)	0.99	0.01 (-0.32 to 0.35)	0.93	0.01 (-0.33 to 0.36)	0.94	0.03 (-0.31 to 0.37)	0.88
Central SBP								
Total n-6 PUFAs	0.13 (-0.07 to 0.33)	0.22	0.12 (-0.08 to 0.33)	0.23	0.12 (-0.09 to 0.33)	0.26	0.17 (-0.04 to 0.38)	0.11
Linoleic acid	0.05 (-0.15 to 0.24)	0.63	0.04 (-0.15 to 0.24)	0.68	0.04 (-0.16 to 0.24)	0.69	0.10 (-0.10 to 0.31)	0.34
Dihomo-ɣ-linolenic acid	0.52 (-0.04 to 1.07)	0.07	0.51 (-0.04 to 1.06)	0.07	0.54 (-0.03 to 1.10)	0.06	0.46 (-0.12 to 1.04)	0.12
Arachidonic acid	-0.03 (-0.44 to 0.38)	0.89	-0.01 (-0.42 to 0.39)	0.97	-0.04 (-0.46 to 0.37)	0.83	0.00 (-0.42 to 0.41)	0.99
Central PP								
Total n-6 PUFAs	0.05 (-0.09 to 0.19)	0.50	0.05 (-0.09 to 0.19)	0.50	0.05 (-0.09 to 0.19)	0.50	0.05 (-0.10 to 0.20)	0.52
Linoleic acid	0.03 (-0.11 to 0.17)	0.66	0.03 (-0.11 to 0.17)	0.67	0.04 (-0.10 to 0.18)	0.57	0.03 (-0.12 to 0.17)	0.70
Dihomo-ɣ-linolenic acid	0.22 (-0.16 to 0.61)	0.26	0.22 (-0.16 to 0.61)	0.26	0.21 (-0.18 to 0.60)	0.29	0.26 (-0.15 to 0.66)	0.21
Arachidonic acid	-0.03 (-0.31 to 0.26)	0.85	-0.02 (-0.31 to 0.26)	0.87	-0.06 (-0.35 to 0.23)	0.69	-0.03 (-0.32 to 0.26)	0.83

CI= confidence interval, PC= phosphatidylcholine, PUFAs= polyunsaturated fatty acids, MUFAs= monounsaturated fatty acids, SBP= systolic blood pressure, DBP= diastolic blood pressure, PP= pulse pressure.

There were 14 women with hypertension before pregnancy. Final model was derived from multiple linear regression with adjustment for maternal age, ethnicity education, exercise, alcohol intake, smoking status, BMI and height at 26 - 28 Weeks gestation, gestational diabetes, heart rate and fish oil supplementation.

Supplemental Table 4-6. Association Between Maternal Plasma PC n-6 PUFAs and Blood Pressures at 26 - 28 Weeks Gestation in Women without Fish Oil Supplementation

	Fatty Acids (%)							
	1st Tertile	2nd Tertile		3rd Tertile		*P*	per 1% Increase	
		β (95% CI)	*P*	β (95% CI)	*P*	(Trend)	β (95% CI)	*P*
Peripheral SBP								
Total n-6 PUFAs	Reference	0.24 (-2.18 to 2.67)	0.84	1.54 (-0.86 to 3.94)	0.21	0.19	0.24 (0.04 to 0.53)	0.10
Linoleic acid	Reference	0.97 (-1.37 to 3.32)	0.42	0.24 (-2.22 to 2.70)	0.85	0.83	0.13 (-0.16 to 0.43)	0.37
Dihomo-γ-linolenic acid	Reference	4.52 (1.99 to 7.04)	<0.001	3.94 (1.45 to 6.34)	0.002	0.005	0.78 (-0.05 to 1.61)	0.06
Arachidonic acid	Reference	1.85 (-0.51 to 4.22)	0.12	1.43 (-1.13 to 3.99)	0.27	0.25	-0.01 (-0.61 to 0.59)	0.97
Peripheral DBP								
Total n-6 PUFAs	Reference	1.08 (-0.89 to 3.05)	0.28	1.38 (-0.56 to 3.33)	0.16	0.17	0.08 (-0.16 to 0.31)	0.53
Linoleic acid	Reference	1.01 (-0.89 to 2.91)	0.29	-0.01 (-2.01 to 1.99)	0.99	0.97	-0.02 (-0.25 to 0.22)	0.89
Dihomo-γ-linolenic acid	Reference	2.67 (0.59 to 4.74)	0.01	1.52 (-0.52 to 3.56)	0.14	0.22	-0.01 (-0.68 to 0.67)	0.98
Arachidonic acid	Reference	2.23 (0.32 to 4.15)	0.02	1.83 (-0.24 to 3.90)	0.08	0.07	0.30 (-0.19 to 0.79)	0.23
Central SBP								
Total n-6 PUFAs	Reference	0.25 (-2.02 to 2.52)	0.83	1.29 (-0.96 to 3.54)	0.26	0.25	0.19 (-0.08 to 0.47)	0.16
Linoleic acid	Reference	1.49 (-0.69 to 3.67)	0.18	-0.64 (-2.93 to 1.66)	0.59	0.63	0.04 (-0.24 to 0.31)	0.79
Dihomo-γ-linolenic acid	Reference	3.82 (1.45 to 6.19)	0.002	3.36 (1.03 to 5.69)	0.005	0.01	0.62 (-0.16 to 1.39)	0.12
Arachidonic acid	Reference	2.80 (0.60 to 4.99)	0.01	2.29 (-0.09 to 4.68)	0.06	0.05	0.28 (-0.28 to 0.84)	0.32
Central PP								
Total n-6 PUFAs	Reference	-0.83 (-2.40 to 0.74)	0.29	-0.09 (-1.64 to 1.45)	0.90	0.97	0.12 (-0.07 to 0.31)	0.21
Linoleic acid	Reference	0.47 (-1.04 to 1.98)	0.54	-0.63 (-2.22 to 0.96)	0.44	0.46	0.05 (-0.14 to 0.24)	0.58
Dihomo-γ-linolenic acid	Reference	1.15 (-0.50 to 2.79)	0.17	1.84 (0.22 to 3.47)	0.03	0.03	0.63 (0.09 to 1.16)	0.02
Arachidonic acid	Reference	0.56 (-0.96 to 2.09)	0.47	0.46 (-1.19 to 2.12)	0.59	0.57	-0.02 (-0.40 to 0.37)	0.93

CI= confidence interval, PC= phosphatidylcholine, PUFAs= polyunsaturated fatty acids, MUFAs= monounsaturated fatty acids, SBP= systolic blood pressure, DBP= diastolic blood pressure, PP= pulse pressure.

Subgroup analysis was performed in 383 women without fish oil supplementation. Results were derived from multiple linear regression with adjustment for maternal age, ethnicity, education, exercise, alcohol intake, smoking status, BMI and height at 26 - 28 weeks gestation, gestational diabetes and heart rate.

Supplemental Table 4-7. Association Between Maternal Plasma PC n-3 PUFA and Blood Pressures at 26 - 28 Weeks Gestation: Stratified by Ethnicity

Fatty Acids (%)	Maternal Ethnicity	Blood Pressure Outcomes								
		1st Tertile	2nd Tertile		3rd Tertile			per 1% Increase		
			β (95% CI)	*P*	β (95% CI)	*P*	*P* (Trend)	β (95% CI)	*P*	*P* (Interaction)
Peripheral SBP										
Total n-3 PUFAs	Chinese	Reference	-3.84 (-6.35 to -1.34)	0.003	-3.15 (-5.53 to -0.77)	0.01	0.02	-0.66 (-1.17 to -0.15)	0.01	0.06
	Malay	Reference	0.07 (-2.98 to 3.12)	0.96	-0.43 (-4.03 to 3.16)	0.81	0.83	0.21 (-0.60 to 1.01)	0.61	
	Indian	Reference	-4.46 (-8.21 to -0.71)	0.02	-3.44 (-7.68 to 0.80)	0.11	0.07	-0.85 (-1.76 to 0.06)	0.07	
α-linolenic acid	Chinese	Reference	1.60 (-0.87 to 4.07)	0.20	-0.44 (-2.81 to 1.92)	0.71	0.69	-1.63 (-9.13 to 5.86)	0.67	0.69
	Malay	Reference	1.96 (-1.25 to 5.18)	0.23	2.29 (-1.24 to 5.84)	0.20	0.18	3.64 (-7.07 to 14.35)	0.50	
	Indian	Reference	1.74 (-2.34 to 5.83)	0.40	-1.03 (-5.10 to 3.03)	0.62	0.54	-0.22 (-10.68 to 10.25)	0.97	
Long chain n-3 PUFAs	Chinese	Reference	-3.29 (-5.78 to -0.80)	0.01	-3.62 (-5.99 to -1.25)	0.003	0.004	-0.65 (-1.18 to -0.13)	0.02	0.07
	Malay	Reference	0.72 (-2.33 to 3.77)	0.64	1.50 (-2.11 to 5.08)	0.42	0.41	0.20 (-0.65 to 1.05)	0.64	
	Indian	Reference	-4.29 (-8.19 to -0.39)	0.03	-2.49 (-6.67 to 1.69)	0.24	0.18	-0.90 (-1.85 to 0.05)	0.06	
n-3/n-6 ratio*	Chinese	Reference	-1.80 (-4.32 to 0.72)	0.16	-2.69 (-5.1 to -0.28)	0.03	0.03	-1.84 (-3.38 to -0.31)	0.02	0.02
	Malay	Reference	-0.94 (-3.98 to 2.11)	0.55	-0.54 (-4.09 to 3.02)	0.77	0.71	0.57 (-1.72 to 2.86)	0.62	
	Indian	Reference	-3.10 (-7.09 to 0.89)	0.13	-4.76 (-8.93 to -0.60)	0.03	0.02	-3.45 (-6.17 to -0.82)	0.01	
Peripheral DBP										
Total n-3 PUFAs	Chinese	Reference	-2.00 (-3.94 to -0.06)	0.04	-1.36 (-3.20 to 0.48)	0.15	0.18	-0.27 (-0.67 to 0.12)	0.17	0.03
	Malay	Reference	0.97 (-1.33 to 3.26)	0.41	1.02 (-1.68 to 3.73)	0.46	0.41	0.51 (-0.10 to 1.11)	0.10	
	Indian	Reference	-1.56 (-4.54 to 1.42)	0.30	-1.34 (-4.71 to 2.03)	0.43	0.38	-0.39 (-1.11 to 0.33)	0.28	
α-linolenic acid	Chinese	Reference	1.59 (-0.30 to 3.48)	0.10	-0.70 (-2.51 to 1.11)	0.45	0.42	-3.75 (-9.49 to 2.00)	0.20	0.19
	Malay	Reference	-0.29 (-2.71 to 2.14)	0.82	1.44 (-1.24 to 4.11)	0.29	0.33	3.99 (-4.08 to 12.06)	0.33	
	Indian	Reference	0.53 (-2.68 to 3.74)	0.74	-0.22 (-3.41 to 2.97)	0.89	0.86	0.14 (-8.01 to 8.29)	0.97	

Long chain n-3 PUFAs	Chinese	Reference	-1.60 (-3.53 to 0.33)	0.10	-1.58 (-3.41 to 0.26)	0.09	0.11	-0.26 (-0.66 to 0.15)	0.22	0.04
	Malay	Reference	0.56 (-1.73 to 2.85)	0.63	2.42 (-0.27 to 5.12)	0.08	0.09	0.51 (-0.13 to 1.14)	0.12	
	Indian	Reference	-1.80 (-4.89 to 1.28)	0.25	-1.14 (-4.44 to 2.17)	0.50	0.44	-0.41 (-1.15 to 0.34)	0.28	
n-3/n-6 ratio*	Chinese	Reference	-1.06 (-3.00 to 0.88)	0.29	-0.99 (-2.85 to 0.87)	0.30	0.32	-0.54 (-1.73 to 0.64)	0.37	0.03
	Malay	Reference	-0.09 (-2.39 to 2.21)	0.94	0.92 (-1.77 to 3.60)	0.50	0.55	1.42 (-0.29 to 3.14)	0.10	
	Indian	Reference	-2.31 (-5.44 to 0.81)	0.15	-3.16 (-6.42 to 0.10)	0.06	0.05	-1.82 (-3.94 to 0.29)	0.09	
Central SBP										
Total n-3 PUFAs	Chinese	Reference	-2.79 (-5.12 to -0.46)	0.02	-2.56 (-4.78 to -0.35)	0.02	0.03	-0.49 (-0.97 to -0.02)	0.04	0.02
	Malay	Reference	1.05 (-1.76 to 3.87)	0.46	0.38 (-2.93 to 3.70)	0.82	0.74	0.46 (-0.28 to 1.2)	0.23	
	Indian	Reference	-3.76 (-7.22 to -0.29)	0.03	-2.99 (-6.90 to 0.93)	0.13	0.09	-0.67 (-1.51 to 0.17)	0.12	
α-linolenic acid	Chinese	Reference	1.53 (-0.75 to 3.82)	0.19	-1.10 (-3.29 to 1.09)	0.32	0.31	-3.63 (-10.57 to 3.31)	0.30	0.42
	Malay	Reference	1.67 (-1.30 to 4.64)	0.27	1.89 (-1.39 to 5.16)	0.26	0.24	3.67 (-6.22 to 13.56)	0.47	
	Indian	Reference	1.65 (-2.10 to 5.4)	0.39	-1.33 (-5.06 to 2.39)	0.48	0.41	-0.49 (-10.12 to 9.14)	0.92	
Long chain n-3 PUFAs	Chinese	Reference	-2.44 (-4.76 to -0.12)	0.04	-2.85 (-5.06 to -0.64)	0.01	0.01	-0.46 (-0.95 to 0.04)	0.07	0.03
	Malay	Reference	1.12 (-1.69 to 3.93)	0.43	2.52 (-0.78 to 5.82)	0.13	0.13	0.47 (-0.31 to 1.25)	0.23	
	Indian	Reference	-3.77 (-7.36 to -0.17)	0.04	-2.20 (-6.05 to 1.66)	0.26	0.20	-0.72 (-1.60 to 0.16)	0.11	
n-3/n-6 ratio*	Chinese	Reference	-1.04 (-3.38 to 1.30)	0.38	-2.01 (-4.25 to 0.23)	0.08	0.08	-1.36 (-2.79 to 0.06)	0.06	0.02
	Malay	Reference	0.06 (-2.76 to 2.88)	0.97	0.62 (-2.66 to 3.91)	0.71	0.73	1.14 (-0.97 to 3.25)	0.29	
	Indian	Reference	-2.61 (-6.30 to 1.07)	0.16	-4.00 (-7.85 to -0.15)	0.04	0.04	-2.57 (-5.06 to -0.08)	0.04	
Central PP										
Total n-3 PUFAs	Chinese	Reference	-0.79 (-2.35 to 0.76)	0.32	-1.20 (-2.68 to 0.28)	0.11	0.11	-0.22 (-0.54 to 0.09)	0.17	0.67
	Malay	Reference	0.09 (-1.79 to 1.96)	0.93	-0.64 (-2.85 to 1.57)	0.57	0.61	-0.05 (-0.55 to 0.45)	0.84	
	Indian	Reference	-2.20 (-5.13 to 0.73)	0.14	-1.65 (-4.96 to 1.67)	0.33	0.27	-0.28 (-0.99 to 0.43)	0.43	
α-linolenic acid	Chinese	Reference	-0.06 (-1.58 to 1.47)	0.94	-0.40 (-1.86 to 1.06)	0.59	0.59	0.11 (-4.49 to 4.72)	0.96	0.91
	Malay	Reference	1.96 (-0.01 to 3.92)	0.05	0.45 (-1.72 to 2.62)	0.68	0.56	-0.32 (-6.92 to 6.28)	0.92	
	Indian	Reference	1.12 (-2.03 to 4.27)	0.48	-1.11 (-4.24 to 2.02)	0.48	0.42	-0.63 (-8.70 to 7.44)	0.88	

Long chain n-3 PUFAs	Chinese	Reference	-0.84 (-2.39 to 0.70)	0.28	-1.27 (-2.75 to 0.20)	0.09	0.09	-0.20 (-0.53 to 0.13)	0.23	
	Malay	Reference	0.56 (-1.32 to 2.44)	0.56	0.10 (-2.12 to 2.31)	0.93	0.87	-0.04 (-0.56 to 0.49)	0.89	0.70
	Indian	Reference	-1.96 (-5.01 to 1.09)	0.21	-1.06 (-4.33 to 2.21)	0.52	0.45	-0.31 (-1.05 to 0.43)	0.41	
n-3/n-6 ratio*	Chinese	Reference	0.02 (-1.53 to 1.57)	0.98	-1.02 (-2.51 to 0.46)	0.18	0.15	-0.82 (-1.76 to 0.12)	0.09	
	Malay	Reference	0.16 (-1.72 to 2.03)	0.87	-0.29 (-2.48 to 1.90)	0.79	0.83	-0.28 (-1.69 to 1.13)	0.69	0.66
	Indian	Reference	-0.30 (-3.45 to 2.85)	0.85	-0.84 (-4.13 to 2.44)	0.61	0.62	-0.74 (-2.86 to 1.37)	0.49	

CI= confidence interval, PC= phosphatidylcholine, PUFAs= polyunsaturated fatty acids, MUFAs= monounsaturated fatty acids, SBP= systolic blood pressure, DBP= diastolic blood pressure, PP= pulse pressure.

*The values were β (95% CI) for blood pressures in 0.1-unit increase of the n-3/n-6 ratio.

Results were derived from multiple linear regression with adjustment for maternal age, education, exercise, alcohol intake, smoking status, BMI and height at 26 - 28 weeks gestation, gestational diabetes, heart rate and fish oil supplementation. Study sample comprised of 404 Chinese, 217 Malay and 130 Indian women

Supplemental Table 4-8. Association Between Maternal Plasma PC n-6 PUFAs and Blood Pressures at 26 - 28 Weeks Gestation: Stratified by Ethnicity

Fatty Acids (%)	Maternal Ethnicity	Blood Pressure Outcomes								
		1st Tertile	2nd Tertile		3rd Tertile			per 1% Increase		
			β (95% CI)	*P*	β (95% CI)	*P*	*P* Trend	β (95% CI)	*P*	*P* Interaction
Peripheral SBP										
Total n-6 PUFAs	Chinese	Reference	-0.68 (-3.03 to 1.68)	0.57	1.22 (-1.26 to 3.70)	0.33	0.36	0.11 (-0.21 to 0.44)	0.49	0.14
	Malay	Reference	-3.09 (-6.28 to 0.09)	0.06	0.41 (-3.01 to 3.83)	0.81	0.79	0.13 (-0.30 to 0.57)	0.55	
	Indian	Reference	3.73 (-0.62 to 8.09)	0.09	5.33 (1.24 to 9.42)	0.01	0.01	0.52 (0.09 to 0.95)	0.02	
Linoleic acid	Chinese	Reference	1.45 (-0.92 to 3.83)	0.23	1.34 (-1.06 to 3.75)	0.27	0.27	0.04 (-0.26 to 0.35)	0.78	0.02
	Malay	Reference	-0.82 (-4.33 to 2.68)	0.64	0.24 (-3.05 to 3.54)	0.88	0.83	0.11 (-0.29 to 0.50)	0.59	
	Indian	Reference	4.49 (1.03 to 7.94)	0.01	6.27 (2.04 to 10.51)	0.004	0.002	0.77 (0.27 to 1.27)	0.003	
Dihomo-ɣ-linolenic acid	Chinese	Reference	2.63 (0.33 to 4.93)	0.03	1.63 (-0.88 to 4.16)	0.20	0.14	0.47 (-0.41 to 1.33)	0.30	0.97
	Malay	Reference	1.73 (-1.76 to 5.23)	0.33	2.53 (-0.95 to 6.00)	0.15	0.16	0.83 (-0.36 to 2.02)	0.17	
	Indian	Reference	1.26 (-3.90 to 6.41)	0.63	2.99 (-1.65 to 7.63)	0.21	0.16	0.35 (-0.92 to 1.61)	0.59	
Arachidonic acid	Chinese	Reference	1.17 (-1.17 to 3.51)	0.33	0.97 (-1.55 to 3.48)	0.45	0.44	-0.05 (-0.70 to 0.60)	0.88	0.71
	Malay	Reference	-0.70 (-3.72 to 2.33)	0.65	-2.96 (-6.74 to 0.83)	0.13	0.15	-0.54 (-1.49 to 0.42)	0.27	
	Indian	Reference	-1.15 (-6.50 to 4.20)	0.67	0.04 (-4.52 to 4.59)	0.99	0.84	-0.24 (-1.06 to 0.58)	0.56	
Peripheral DBP										
Total n-6 PUFAs	Chinese	Reference	-0.42 (-2.23 to 1.39)	0.65	-0.13 (-2.04 to 1.78)	0.89	0.87	-0.06 (-0.32 to 0.19)	0.61	0.05
	Malay	Reference	-0.56 (-2.98 to 1.87)	0.65	1.28 (-1.33 to 3.88)	0.34	0.33	0.07 (-0.26 to 0.40)	0.68	
	Indian	Reference	2.22 (-1.20 to 5.64)	0.20	3.52 (0.31 to 6.73)	0.03	0.03	0.37 (0.03 to 0.71)	0.03	
Linoleic acid	Chinese	Reference	0.06 (-1.77 to 1.89)	0.95	0.10 (-1.75 to 1.96)	0.91	0.91	-0.07 (-0.30 to 0.16)	0.56	0.13
	Malay	Reference	1.42 (-1.21 to 4.05)	0.29	-0.09 (-2.56 to 2.38)	0.94	0.84	0.06 (-0.24 to 0.36)	0.68	
	Indian	Reference	1.81 (-0.96 to 4.59)	0.19	2.73 (-0.68 to 6.14)	0.12	0.09	0.31 (-0.09 to 0.71)	0.13	

Dihomo-γ-linolenic acid	Chinese	Reference	1.66 (-0.11 to 3.43)	0.07	1.16 (-0.78 to 3.10)	0.24	0.18	0.22 (-0.45 to 0.89)	0.51	0.49
	Malay	Reference	0.76 (-1.88 to 3.41)	0.57	1.32 (-1.31 to 3.95)	0.32	0.32	0.17 (-0.74 to 1.07)	0.72	
	Indian	Reference	2.44 (-1.55 to 6.43)	0.23	3.37 (-0.22 to 6.97)	0.07	0.07	0.60 (-0.38 to 1.58)	0.23	
Arachidonic acid	Chinese	Reference	1.54 (-0.25 to 3.33)	0.09	0.02 (-1.90 to 1.94)	0.98	0.95	-0.15 (-0.65 to 0.35)	0.55	0.60
	Malay	Reference	0.02 (-2.27 to 2.32)	0.98	-0.87 (-3.74 to 2.01)	0.55	0.61	-0.19 (-0.92 to 0.53)	0.60	
	Indian	Reference	2.21 (-1.94 to 6.36)	0.29	1.96 (-1.58 to 5.49)	0.28	0.37	0.27 (-0.36 to 0.91)	0.40	
Central SBP										
Total n-6 PUFAs	Chinese	Reference	-0.70 (-2.89 to 1.48)	0.53	0.58 (-1.72 to 2.88)	0.62	0.66	0.05 (-0.25 to 0.36)	0.73	0.26
	Malay	Reference	-2.34 (-5.30 to 0.61)	0.12	0.67 (-2.50 to 3.84)	0.68	0.66	0.17 (-0.24 to 0.57)	0.42	
	Indian	Reference	1.37 (-2.70 to 5.44)	0.51	3.21 (-0.61 to 7.03)	0.10	0.09	0.30 (-0.10 to 0.70)	0.14	
Linoleic acid	Chinese	Reference	1.21 (-0.99 to 3.42)	0.28	0.44 (-1.79 to 2.67)	0.69	0.69	-0.02 (-0.30 to 0.26)	0.89	0.11
	Malay	Reference	-0.17 (-3.40 to 3.06)	0.92	-0.43 (-3.46 to 2.61)	0.78	0.78	0.08 (-0.29 to 0.45)	0.68	
	Indian	Reference	3.87 (0.64 to 7.09)	0.02	3.97 (0.01 to 7.93)	0.05	0.02	0.39 (-0.08 to 0.87)	0.10	
Dihomo-γ-linolenic acid	Chinese	Reference	2.11 (-0.02 to 4.25)	0.05	1.79 (-0.55 to 4.13)	0.13	0.10	0.40 (-0.40 to 1.21)	0.33	0.99
	Malay	Reference	1.19 (-2.04 to 4.41)	0.47	2.50 (-0.70 to 5.71)	0.13	0.12	0.79 (-0.31 to 1.89)	0.16	
	Indian	Reference	2.41 (-2.32 to 7.14)	0.32	3.48 (-0.78 to 7.74)	0.11	0.12	0.37 (-0.79 to 1.54)	0.53	
Arachidonic acid	Chinese	Reference	1.56 (-0.60 to 3.72)	0.16	0.83 (-1.49 to 3.16)	0.48	0.47	0.03 (-0.57 to 0.63)	0.93	0.93
	Malay	Reference	-0.13 (-2.93 to 2.68)	0.93	-1.37 (-4.88 to 2.15)	0.44	0.50	-0.19 (-1.07 to 0.70)	0.68	
	Indian	Reference	-0.19 (-5.12 to 4.74)	0.94	0.36 (-3.83 to 4.56)	0.86	0.81	-0.11 (-0.86 to 0.65)	0.78	
Central PP										
Total n-6 PUFAs	Chinese	Reference	-0.28 (-1.73 to 1.16)	0.70	0.71 (-0.81 to 2.24)	0.36	0.39	0.12 (-0.08 to 0.32)	0.25	0.83
	Malay	Reference	-1.79 (-3.76 to 0.19)	0.08	-0.61 (-2.73 to 1.51)	0.57	0.59	0.10 (-0.17 to 0.37)	0.48	
	Indian	Reference	-0.84 (-4.30 to 2.61)	0.63	-0.31 (-3.55 to 2.94)	0.85	0.93	-0.07 (-0.41 to 0.27)	0.69	
	Chinese	Reference	1.14 (-0.31 to 2.61)	0.12	0.34 (-1.14 to 1.81)	0.65	0.64	0.05 (-0.14 to 0.24)	0.59	0.79

Linoleic acid	Malay	Reference	-1.59 (-3.74 to 0.55)	0.14	-0.34 (-2.34 to 1.67)	0.74	0.86	0.01 (-0.23 to 0.26)	0.91	
	Indian	Reference	2.05 (-0.71 to 4.81)	0.14	1.24 (-2.14 to 4.62)	0.47	0.33	0.08 (-0.32 to 0.49)	0.68	
Dihomo-γ-linolenic acid	Chinese	Reference	0.45 (-0.97 to 1.87)	0.53	0.63 (-0.93 to 2.19)	0.43	0.41	0.18 (-0.36 to 0.71)	0.51	
	Malay	Reference	0.43 (-1.73 to 2.58)	0.70	1.18 (-0.96 to 3.33)	0.28	0.27	0.62 (-0.11 to 1.35)	0.09	0.29
	Indian	Reference	-0.03 (-4.04 to 3.98)	0.99	0.10 (-3.51 to 3.71)	0.96	0.94	-0.23 (-1.21 to 0.75)	0.64	
Arachidonic acid	Chinese	Reference	0.02 (-1.41 to 1.46)	0.98	0.81 (-0.73 to 2.35)	0.30	0.31	0.18 (-0.22 to 0.58)	0.38	
	Malay	Reference	-0.15 (-2.02 to 1.72)	0.87	-0.50 (-2.84 to 1.85)	0.68	0.69	0.01 (-0.58 to 0.60)	0.98	0.42
	Indian	Reference	-2.39 (-6.50 to 1.71)	0.25	-1.59 (-5.09 to 1.90)	0.37	0.54	-0.38 (-1.01 to 0.25)	0.23	

CI= confidence interval, PC= phosphatidylcholine, PUFAs= polyunsaturated fatty acids, MUFAs= monounsaturated fatty acids, SBP= systolic blood pressure, DBP= diastolic blood pressure, PP= pulse pressure.

Results were derived from multiple linear regression with adjustment for maternal age, education, exercise, alcohol intake, smoking status, BMI and height at 26 - 28 weeks gestation, gestational diabetes, heart rate and fish oil supplementation. Study sample comprised of 404 Chinese, 217 Malay and 130 Indian women.

CHAPTER 5

MATERNAL BLOOD PRESSURES DURING PREGNANCY AND OFFSPRING SIZE AT BIRTH

This chapter was based on the paper published in BMC Pregnancy and Childbirth:

Wai-Yee Lim, Yung-Seng Lee, Chuen-Seng Tan, Kenneth Kwek, Yap-Seng Chong, Peter D Gluckman, Keith M Godfrey, Seang-Mei Saw and An Pan. The association between maternal blood pressures and offspring size at birth in Southeast Asian women. *BMC Pregnancy Childbirth*. 2014 Dec 2;14(1):403.

5.1 Introduction

Birth weight is an important measure of intra-uterine growth. Various maternal and fetal factors are known to influence size at birth. [69, 73] Amongst these factors, maternal blood pressures have been considered as an important determinant. Various epidemiological studies have suggested that maternal hypertension is associated with an increased risk of lower birth weight.[150, 151] Reduced utero-placental function has been suggested as one possible mechanism because this has been found to occur in women with concurrent pre-eclampsia and fetal growth restriction.[73]

Several studies have investigated the associations between offspring's birth weight and maternal peripheral [9, 118, 150, 152-165] and central blood pressures,[155, 159, 160] with inverse relations reported in most studies,[9, 150, 152-155, 159-165] but not all (Table 5-1).[156-158] Some studies have also suggested that the relation between maternal central blood pressures and size at birth may be more pronounced than peripheral blood pressures.[159, 160] However, studies that compared maternal peripheral and central blood pressures concurrently were fewer and smaller in sample size, ranging around 40-50 women, compared to studies that examined peripheral blood pressures only.[155, 159, 160]

Table 5-1: Evidence Table for the Associations between Maternal Blood Pressures, Hypertension and Birth Weight

ID	Author/Year	Design	Sample size/ Country	Gestational Age at Blood Pressure Measurements	Measures of Blood Pressures	Results
1	Tonimatsu et al, 2013	Prospective cohort	40/ Japan	At 26 – 33 weeks gestation	Central SBP and augmentation index Peripheral SBP, DBP and PP	Central SBP and augmentation index were inversely associated with birth weight (r=-0.26, p<0.01 and r=-0.33, p<0.01, respectively). Peripheral SBP, DBP and PP were not associated with birth weight.
2	Khan et al, 2010	Prospective cohort	50/ United Kingdom	At 22 and 34 weeks gestation	Central augmentation index	Central augmentation index, measured at 22 and 34 weeks gestation was inversely correlated with birth weight centile (r=-0.36, p=0.01 and r=-0.42, p=0.05, respectively)
3	Bakker et al, 2011	Prospective cohort	8862/ Netherlands	At pregnancy trimesters; (9.8-17.6, 18.5-23.6, 28.4-32.9) weeks	Peripheral SBP and DBP	Third trimester DBP is inversely associated with birth weight, length and head circumference, whereas third trimester SBP is inversely associated with lower birth weight only. Second trimester blood pressure is not associated with any birth measures
4	Hilmert et al/ 2007	Prospective cohort	498/ U.S.A	18-20, 24-26 and 32-34 weeks	Peripheral SBP and DBP	Significant interaction between DBP and composite stress (p<0.04); In high stress – negative association between DBP

						and birth weight (p=0.05); no significant association in low stress. No significant association observed in SBP
5	Elvan-Taspinar et al/ 2005	Prospective cohort	50/ Netherlands	26-39 weeks	Pulse wave velocity and central pulse pressure	Pulse wave velocity and central pulse pressure are associated with birth weight (centiles) [ß(SD): -17.6(7.0); p=0.016 and -1.8(0.4); p<0.001, respectively]; and only pulse wave velocity is associated with catch up growth at 6 months [22.3(8.7); p=0.015]
6	Waugh et al/ 2000	Prospective cohort	237/ United Kingdom	Mean gestation 35.6 ± 3.5 weeks	Ambulatory peripheral DBP	Day time blood pressure: SBP: -7.6 (95%CI -15.8 to 0.60) g DBP: -13.5 (95%CI -23.4 to -3.60) g Night time blood pressure: SBP: -4.6 (95%CI -13.5 to 4.30) g DBP: -2.2 (95%CI -9.70 to 5.30) g
7	Churchill et al/ 1997	Prospective cohort	2009/ United Kingdom	At 18, 28 and 38 weeks gestation	Peripheral SBP and DBP	18 weeks gestation: SBP: 0·7 (-9·4 to 10·8) g DBP: -8·1 (-23·3 to 6·9) g 28 weeks gestation: SBP: -4·0 (-14·5 to 6·6) g DBP: -21·5 (-35·7 to -7·2) g 38 weeks gestation:

						SBP: -10·8 (-18·4 to -3·2) g DBP: -24·1 (-33·7 to -14·5) g
8	Yadav et al/ 2013	Retrospective cohort	666/ Malaysia	Across the pregnancy trimesters	Average of peripheral SBP and DBP	Mothers with SBP < 110 mmHg or >130 mmHg had higher risk to LBW compared to those with SBP 110 – 130 mmHg (OR 1.2 and 1.4 respectively) Mothers with DBP <75 mmHg or >85 mmHg had higher risk to LBW compared to those with DBP 75-85 mmHg (OR 1.2 and 1.5, respectively)
9	Makgoba et al/ 2012	Retrospective cohort	130549/ United Kingdom	At Booking (<20 weeks)	Highest peripheral DBP	Positively associated with birth weight (z scores) for South Asians only, p<0.001
10	Steer et al/ 2004	Retrospective cohort	210814/ United Kingdom	At first booking and throughout pregnancy	Peripheral DBP	>34 weeks gestation, Inverted u shaped association between DBP and birth weight. Highest birth weight was observed in DBP between 70-90mmHg
11	Zhang et al, M.A./ 2001	Retrospective cohort	58760/ United States of America	From prenatal at <25 weeks to postnatal <45 weeks	Peripheral DBP	High DBP at baseline and excessive rise ≥15mmHg is associated with twice the risk of having a small baby
12	Xiong et al/ 2004	Retrospective cohort	16936/ China	Diagnosed after 20 weeks gestation	Gestational hypertension and preeclampsia	Significant difference in birth weight between women with severe preeclampsia vs normotensive women when delivered at <35 and 36 weeks gestation, at -467.7 g and -294.6 g, respectively

						Women with gestational hypertension/ preeclampsia had higher risk to delivering small for gestational age offspring compared to women with normotensive pregnancies
13	Odegard et al/ 2000	Retrospective cohort	1016/ Norway	Diagnosed after 20 weeks gestation	Preeclampsia	Risk of SGA was 4 times higher in women with preeclampsia (RR 4.2 95%CI 2.2 – 8.0)
14	Lydakis et al/ 1998	Retrospective cohort	436/ United Kingdom	Diagnosed after 20 weeks gestation	Preeclampsia	Women with preeclampsia had higher risk to smaller offspring (birth weight < 2kg); In whites: RR 2.8 (95%CI 1.6 – 5.0) In blacks: RR 2.8 (95%CI 1.7 – 4.5) In Indo-Asians: RR 5.6 (95%CI 3.8 – 8.2)
15	Dhall et al/ 1995	Retrospective cohort	3293/ India	Between 33 – 41 weeks gestation	Pregnancy induced hypertension	Severe PIH were associated with significant lowering of birth weight. SBP ≥160 mm of Hg : Birth weight reduced by 151 g ($p<0.01$) DBP ≥ 110 mm of Hg : Birth weight reduced by 216 g ($p<0.001$)
16	Romundstad et al/2007	Cross-sectional/ Linkage study	3461/ Norway	Pre-pregnancy	Peripheral SBP and DBP	Women with higher pre-pregnancy peripheral SBP and DBP tended to deliver smaller babies.

17	Maggioni et al/ 2005	Cross-sectional	52/ Italy	33 weeks	24hrs ambulatory peripheral SBP and DBP	DBP amplitude (extent of change within the day) is associated with increased odds to growth restricted newborns OR 1.7 (95%CI 1.1–2.8), p=0.03; SBP amplitude and mean (SBP & DBP) not associated with growth restriction

SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; OR, odds ratio; RR, risk ratio

There is evidence that the relation between maternal blood pressures and offspring's birth weight were found to be stronger in Asian Indians than white or black women, [161] and was more evident in normal weight women than obese women.[152] As the incidence of small for gestational age in Southeast Asian women is one of the highest in the world,[70] examining inter-ethnic variation may enable specific and appropriate public health interventions.

Therefore, we aimed to simultaneously examine both maternal peripheral and central blood pressures in relation to size at birth, and to explore the possible effect modification by maternal ethnicity or adiposity in pregnancy in a Southeast Asian birth cohort of pregnant Chinese, Malay and Indian women.

5.2 Methods

The present study sample was drawn from the **G**rowing **U**p in **S**ingapore **T**owards healthy **O**utcomes (GUSTO) study,[85] a prospective early life cohort study comprising Chinese, Indian and Malay women. Between 2009 and 2010, a total of 1162 pregnant women without type 1 diabetes or using chemotherapy or psychotropic drugs were recruited from two tertiary hospitals in Singapore. We excluded 333 women (28.6%) women who did not attend the blood pressure measurements or had incomplete recordings around 27 weeks, and 116 women who had incomplete demographic and pregnancy information, leaving a total of 713 women for the current analysis (Figure 5-1). Women who were excluded from the analysis had similar demographic characteristics compared with those who were included, although they had shorter gestation duration and smaller offspring (Supplemental Table 5-1). The

GUSTO study was approved by the SingHealth Centralized Institutional Review Board (CIRB Ref: 2009/280/D) and National Health Group Domain Specific Review Board (DSRB Ref: 09/021), and all participants have given informed consents.

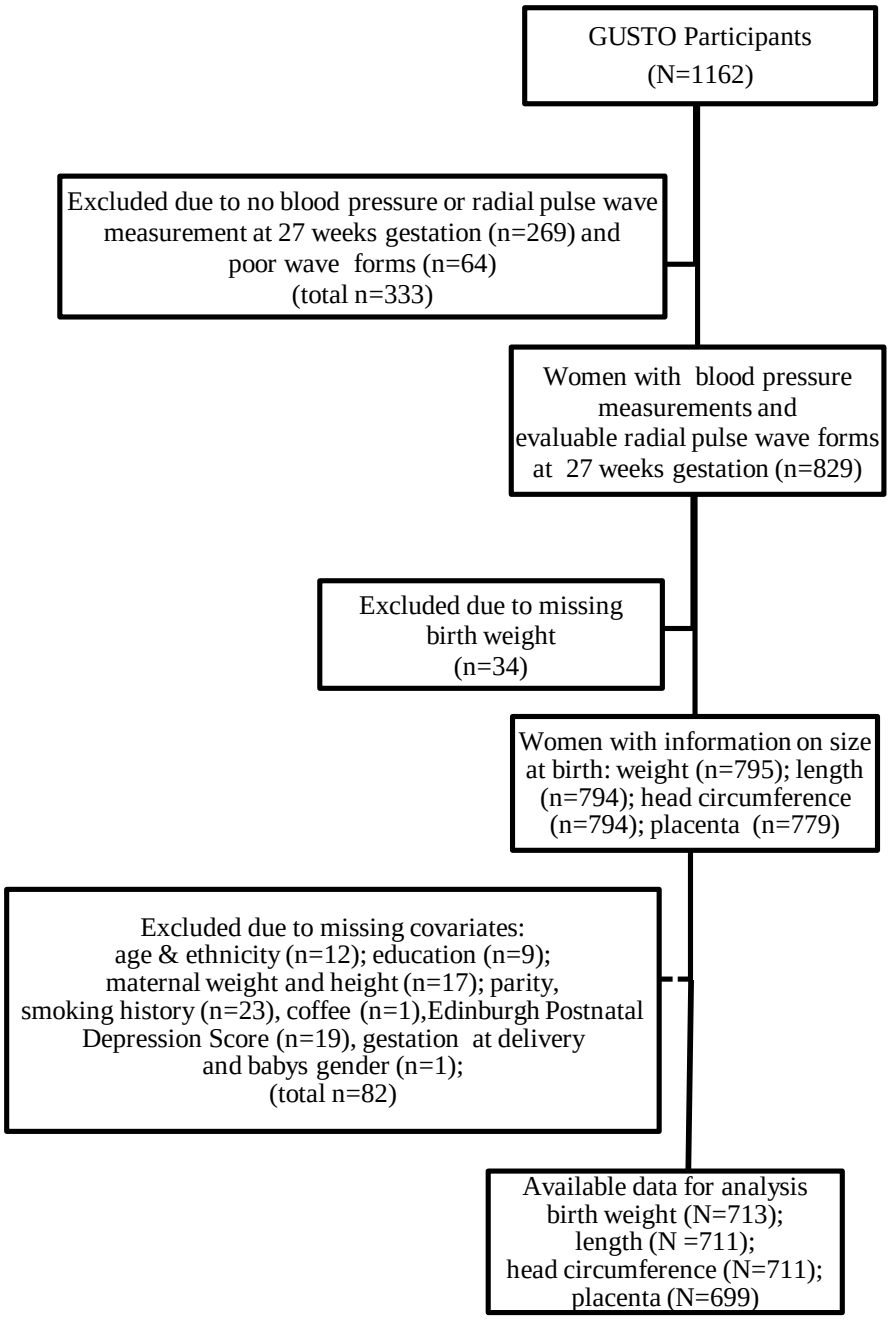


Figure 5-1. Flow Chart of the GUSTO Study Sample Selected for Analysis

At their enrolment visit prior to 14 weeks gestation, study participants were interviewed for baseline information on age, ethnicity, educational level, pre-pregnancy body weight, smoking history, coffee consumption and number of previous live-births. They were followed up at mid-pregnancy [median gestation of 27 weeks (interquartile range 26 to 29 weeks)] to measure maternal height and weight, as well as blood pressures using standard protocols [12]. Depression status (defined as an overall score of 13 or greater from the self-administered Edinburgh Postnatal Depression Scale) and gestational diabetes (defined as fasting plasma glucose ≥7.0 mmol/L or 2-hour glucose ≥11.1 mmol/L)[166] was also examined during this visit. Maternal body mass index (BMI) before pregnancy and GUSTO mid-pregnancy follow-up visit were calculated as weight in kg divided by the square of height in meter, and categorised as normal weight for BMI <25.0 kg/m^2, overweight as 25.0 to 29.9 kg/m^2 and obese as ≥30.0 kg/m^2 according to the WHO international classification.[105] Rate of weight gain was calculated as the weight difference before pregnancy and at GUSTO mid-pregnancy follow-up visit in kg divided by the length of gestation during mid-pregnancy in weeks.

Study participants were rested for at least 10 minutes prior to the first blood pressure measurement. Peripheral systolic and diastolic blood pressures (SBP and DBP) were taken on the right brachial artery at the level of the heart. Using an oscillometric device (MC3100, HealthSTATS International Pte Ltd, Singapore), three blood pressure readings were taken consecutively at 30 to 60 seconds apart to obtain the average reading of SBP and DBP. The A-pulse tonometer (BPro®, HealthSTATS International Pte Ltd, Singapore) was then applied on the radial artery of the same arm for continuous sampling of radial

artery waveforms for at least 60 seconds. Central SBP was estimated from the calibrated radial artery waveforms [12], and central pulse pressure (PP) was calculated as the difference between central SBP and peripheral DBP [62].

Information on offspring size at birth (weight, length, head circumference and placental weight) were extracted from medical records, which were measured by midwives according to standard hospital protocols. Gestational age adjusted standard deviation (SD) scores for birth weight, length, head circumference and placental weight were constructed for the GUSTO cohort. The binary outcome of low birth weight was defined as weight at birth <2500 g, and small for gestational age was defined as those who were below the 10th percentile for gestational age adjusted birth weight.

Blood pressure values were converted into SD scores, whereby per 1-SD increase in peripheral SBP and DBP was equivalent to 11.1 and 8.3 mmHg, respectively, and central SBP and PP to 10.0 and 6.5 mmHg, respectively. Maternal characteristics were compared across blood pressures using analysis of variance. The relation between blood pressures and size at birth were examined using multiple linear and logistic regression models for continuous and binary birth size outcomes, respectively. All analyses were adjusted for baby's sex, gestation age at delivery in weeks, maternal age, ethnicity, education, parity, smoking history, height, BMI around 27 weeks gestation, coffee consumption and depression. No adjustment was made for chronic hypertension as there were only 13 (1.8%) women with this condition.

We further evaluated the potential effect modification by maternal ethnicity (Chinese, Indian, or Malay) and BMI categories (normal, overweight, or obese).

Multiplicative interaction terms with blood pressures as continuous variable and ethnicity or BMI as a categorical variable were added to the final model, and the likelihood ratio test was used to evaluate significance. We also reported the results stratified by maternal ethnicity (Chinese, Indian, or Malay) and BMI category (normal weight, overweight, or obese).

We performed a series of sensitivity analyses to test the robustness of our results: (1) we additionally adjusted for gestational diabetes (n = 663 due to 50 missing data for gestational diabetes status); (2) we examined a subgroup of 705 women within normal range of blood pressures (peripheral SBP and DBP below 140 and 90 mmHg, respectively); (3) we repeated our analysis using the imputed data (20 sets) for the missing information on blood pressures (imputed based on maternal age, ethnicity, education, parity, gestational diabetes, height and BMI around 27 weeks gestation, and the respective outcome variable); (4) we adjusted for pre-pregnancy BMI and rate of weight gain instead of BMI around 27 weeks gestation (n = 678 due to 35 missing data for pre-pregnancy weight); (5) we used tertiles of BMI around 27 weeks gestation instead of the WHO classification to test for interaction between maternal adiposity and blood pressures; (6) lastly, we examined gestational age adjusted size at birth measures as the outcomes instead of actual values to better account for the effect of gestational duration on size at birth. All analyses were performed using Stata version 11.2 (Statacorp, College Station, Texas), with statistical significance at two-sided p value less than 0.05.

5.3 Results

Of the 713 women studied, 339 (55.9%) were Chinese, 196 (27.5%) were Indians and 118 (16.6%) were Malays. The mean age at enrolment was 30.5 (SD = 5.1) years. At the GUSTO study follow-up, 17.5% were obese at around 27 weeks gestation (Tables 1 and Additional file 1: Table S1). Mean (SD) values for offspring's birth weight, length, head circumference and placental weight were 3113.5 (435.0) g, 48.7 (2.2) cm, 33.4 (1.4) cm, and 585.3 (118.9) g, respectively. Women of Malay ethnicity, lower education levels and higher BMI categories were more likely to have higher peripheral and central blood pressures ($p < 0.01$, Table 5-2).

Table 5-2 Distribution of Maternal Blood Pressures by Maternal Characteristics*

Maternal Characteristics		Peripheral Blood Pressures (mmHg)				Central Blood Pressures (mmHg)			
		SBP		DBP		SBP		PP	
	No (%)	Mean (SD)	*P*	Mean (SD)	*P*	Mean (SD)	*P*	Mean (SD)	*P*
Age at booking (years)			0.54		0.26		0.62		0.36
1st quartile (18–26)	162 (22.7)	110.2 (12.0)		66.5 (8.7)		96.5 (10.6)		30.0 (6.9)	
2nd quartile (27–29)	148 (20.8)	109.6 (10.5)		67.1 (8.4)		96.3 (9.6)		29.3 (5.7)	
3rd quartile (30–33)	204 (28.6)	108.7 (11.0)		65.8 (7.8)		96.4 (9.8)		30.5 (6.7)	
4th quartile (34–46)	199 (27.9)	108.9 (10.7)		67.4 (7.9)		97.5 (9.8)		30.1 (6.4)	
Race			0.001		0.004		0.005		0.34
Chinese	399 (55.9)	108.4 (10.9)		66.3 (8.2)		95.9 (9.9)		29.7 (6.4)	
Indian	118 (16.6)	108.3 (10.8)		65.4 (7.9)		95.9 (9.3)		30.4 (7.1)	
Malay	196 (27.5)	111.8 (11.2)		68.2 (8.3)		98.7 (10.2)		30.4 (6.3)	
Education			0.005				0.001		0.84
Primary-Secondary	229 (32.1)	109.7 (11.5)		67.0 (8.4)	<0.001	97.2 (10.4)		30.2 (6.5)	
Post-Secondary	245 (34.4)	110.7 (11.4)		67.9 (8.2)		98.0 (10.0)		30.1 (6.7)	
Tertiary	239 (33.5)	107.5 (10.2)		64.9 (7.8)		94.8 (9.2)		29.8 (6.3)	
Smoking Status			0.01		0.29		0.14		0.34
Never smoker	618 (86.7)	108.9 (10.9)		66.4 (8.4)		96.5 (9.8)		29.9 (6.4)	
Ever smoker	95 (13.3)	111.9 (11.7)		67.5 (8.5)		98.1 (11.0)		30.6 (6.8)	
Coffee Consumption			0.80		0.45		0.39		0.73
No	369 (51.8)	109.2 (10.9)		66.5 (8.4)		96.4 (9.9)		29.9 (6.8)	
Yes	344 (48.2)	109.4 (11.2)		66.9 (8.0)		97.0 (9.9)		30.1 (6.2)	
Parity			0.47		0.72		0.26		0.17
Nulliparous	311 (43.6)	109.1 (11.0)		66.6 (7.9)		96.2 (9.6)		29.6 (6.7)	
Primiparous	246 (34.5)	108.9 (11.3)		66.4 (8.3)		96.6 (10.2)		30.2 (6.5)	
Multiparous	156 (21.9)	110.3 (10.7)		67.1 (8.5)		97.8 (10.2)		30.7 (6.0)	
Gestational Diabetes**			0.10		0.02		0.02		0.40
No	540 (81.4)	109.1 (10.7)		66.4 (7.9)		96.3 (9.6)		29.9 (6.4)	
Yes	123 (18.6)	110.9 (11.6)		68.2 (8.6)		98.7 (10.7)		30.5 (6.4)	
Pre-pregnancy BMI (kg/m^2)***			<0.001		<0.001		<0.001		0.29
<25.0	518 (76.4)	107.0 (10.3)		65.1 (7.6)		94.7 (9.4)		29.7 (6.5)	
25.0-29.9	113 (16.7)	114 (9.7)		70.9 (8.3)		101.3 (8.8)		30.4 (6.4)	
≥30.0	47 (6.9)	119.4 (10.8)		73.4 (7.2)		104.3 (8.9)		30.9 (6.7)	

Rate of weight gain at 27 weeks kg/week)***			0.005		0.004		0.006	0.80
1st tertile (−0.42 – 0.25)	227 (33.5)	107.8 (11.0)		65.9 (8.6)		95.7 (10.1)	29.8 (6.6)	
2nd tertile (0.26 – 0.37)	218 (32.2)	108.4 (10.2)		65.8 (8.1)		95.6 (9.1)	29.8 (6.6)	
3rd tertile (0.38 – 1.38)	233 (34.5)	110.9 (11.2)		68.1 (7.7)		98.2 (10.0)	30.1 (6.3)	
Second trimester BMI (kg/m^2)			<0.001		<0.001		<0.001	<0.001
<25.0	329 (46.2)	104.7 (9.9)		63.9 (7.7)		92.9 (9.3)	29.0 (6.2)	
25.0-29.9	259 (36.3)	110.9 (9.8)		67.3 (7.4)		98.2 (8.7)	30.8 (6.5)	
≥30.0	125 (17.5)	117.9 (10.6)		72.8 (7.4)		103.8 (9.4)	31.0 (6.7)	
Depression			0.99		0.71		0.46	0.52
Not depressed	631 (88.5)	109.3 (11.2)		66.7 (8.1)		96.8 (10.1)	30.1 (6.5)	
Depressed	82 (11.5)	109.3 (10.1)		66.4 (9.0)		95.9 (8.9)	29.6 (6.1)	

SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

*Data are represented as n (%) or mean (SD) where appropriate. *P* values were derived from analysis of variance.

**There were 50 women with missing values for gestational diabetes.

***There were 35 women with missing values for pre-pregnancy BMI and rate of weight gain.

After adjusting for maternal and fetal covariates, central SBP was inversely associated with all birth measures, and peripheral SBP was inversely associated with birth weight (all $p < 0.05$; Table 5-3). For example, each 1-SD increase (10.0 mmHg) in central SBP was inversely associated with birth weight (−40.52 g; 95% confidence interval [CI] -70.66 to −10.37), birth length (−0.19 cm; −0.36 to −0.03), head circumference (−0.12 cm; −0.23 to −0.02) and placental weight (−11.16 cm; −20.85 to −1.47). One-SD (11.1 mmHg) increase in peripheral SBP was also associated with 35.56 g lower birth weight (95% CI −66.57 to −4.54). The relations between other blood pressure measures and offspring size at birth were in the same direction but not statistically significant. Results were also not materially different in various sensitivity analyses (Supplemental Table 5-2).

Table 5-3 Associations between Blood Pressures (Per 1-SD Increase) and Size at Birth*

Measures of Size at Birth		Peripheral Blood Pressures (mmHg)		Central Blood Pressures (mmHg)	
		SBP (1 SD = 11.1 mmHg)	DBP (1 SD = 8.3 mmHg)	SBP (1 SD = 10.0 mmHg)	PP (1 SD = 6.5 mmHg)
	N	ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)
Weight (g)	713	−35.56 (−66.57 to −4.54)	−25.13 (−55.36 to 5.09)	−40.52 (−70.66 to −10.37)	−24.10 (−51.24 to 3.03)
Length (cm)**	711	−0.16 (−0.32 to 0.01)	−0.10 (−0.27 to 0.06)	−0.19 (−0.36 to −0.03)	−0.14 (−0.28 to 0.01)
Head circumference (cm)**	711	−0.09 (−0.19 to 0.02)	−0.08 (−0.18 to 0.02)	−0.12 (−0.23 to −0.02)	−0.07 (−0.16 to 0.02)
Placental weight (g)***	699	−8.78 (−18.74 to 1.19)	−6.94 (−16.63 to 2.76)	−11.16 (−20.85 to −1.47)	−6.44 (−15.04 to 2.16)

SD, standard deviation; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

* Multiple linear regression models were used with adjustment for baby's sex, gestation at delivery, maternal age, ethnicity, education, parity, smoking history, height, BMI at 27 weeks gestation, coffee consumption and depression.

** There were 2 women with missing information on length and head circumference.

*** There were 14 women with missing information on placental weight.

We found no significant interactions between blood pressures and ethnicity in relation to size at birth (Table 5-4). Stratified results for different ethnic groups showed that the associations between blood pressures (peripheral SBP, DBP and central SBP) and birth weight were significant in Chinese women only, but not significant in Malay or Indian women. However, the 95% CIs were large and tended to overlap among the three ethnic groups. We detected significant interactions between blood pressures and maternal BMI categories in relation to offspring's birth weight, length and head circumference, with stronger associations in normal weight women rather than overweight/obese women (Table 5-5). Similar interactions were observed when tertiles of maternal BMI around 27 weeks gestation were used (Supplemental Table 5-3).

Table 5-4 Associations between Blood Pressures (Per 1-SD Increase) and Size at Birth by Maternal Ethnicity*

Maternal Ethnicity	N	Peripheral Blood Pressures (mmHg)		Central Blood Pressures (mmHg)	
		SBP (1 SD = 11.1 mmHg) ß (95% CI)	DBP (1 SD = 8.3 mmHg) ß (95% CI)	SBP (1 SD = 10.0 mmHg) ß (95% CI)	PP (1 SD = 6.5 mmHg) ß (95% CI)
Weight (g)					
Chinese	399	−49.10 (−89.63 to −8.56)	−37.74 (−76.82 to 1.33)	−52.12 (−91.19 to −13.11)	−26.49 (−62.65 to 9.67)
Indian	118	−17.37 (−98.58 to 63.84)	0.17 (−76.87 to 77.22)	−30.70 (−109.08 to 47.67)	−29.87 (−91.32 to 32.15)
Malay	196	−4.88 (−70.38 to 60.62)	−17.29 (−80.97 to 46.39)	−17.00 (−80.93 to 46.93)	−3.21 (−63.08 to 56.65)
P for interaction		0.96	0.78	0.98	0.96
Length (cm)**					
Chinese	398	−0.11 (−0.35 to 0.12)	−0.14 (−0.37 to 0.08)	−0.16 (−0.39 to 0.06)	−0.05 (−0.26 to 0.16)
Indian	117	−0.14 (−0.54 to -.26)	−0.07 (−0.44 to 0.31)	−0.19 (−0.57 to 0.19)	−0.13 (−0.43 to 0.18)
Malay	196	−0.24 (−0.57 to 0.08)	0.05 (−0.27 to 0.37)	−0.24 (−0.56 to 0.08)	−0.38 (−0.67 to −0.08)
P for interaction		0.16	0.96	0.29	0.09
Head circumference (cm)**					
Chinese	398	−0.13 (−0.28 to 0.01)	−0.13 (−0.27 to 0.00)	−0.17 (−0.31 to −0.04)	−0.08 (−0.21 to 0.05)
Indian	117	−0.04 (−0.32 to 0.23)	0.10 (−0.15 to 0.36)	−0.10 (−0.37 to 0.16)	−0.18 (−0.39 to 0.02)
Malay	196	0.03 (−0.19 to 0.26)	−0.09 (−0.31 to 0.13)	−0.00 (−0.22 to 0.22)	0.09 (−0.11 to 0.30)
P for interaction		0.69	0.81	0.63	0.18
Placenta weight (g)***					
Chinese	392	−8.27 (−21.94 to 5.40)	−6.28 (−19.47 to 6.92)	−11.22 (−24.43 to 1.99)	−7.43 (−19.37 to 4.51)
Indian	115	−11.15 (−37.06 to 14.76)	−5.05 (−29.82 to 19.72)	−13.62 (−38.69 to 11.44)	−8.94 (−28.84 to 10.95)
Malay	192	−9.16 (−28.52 to 10.19)	−12.83 (−31.39 to 5.73)	−11.79 (−30.53 to 6.94)	−1.13 (−18.65 to 16.39)
P for interaction		0.93	0.80	0.89	0.97

SD, standard deviation; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

* Multiple linear regression models were used with adjustment for baby's sex, gestation at delivery, maternal age, education, parity, smoking history, height, BMI at 27 weeks gestation, coffee consumption and depression.

** There were 2 women with missing information on length and head circumference.

*** There were 14 women with missing information on placental weight.

Table 5-5 Associations between Blood Pressures (Per 1-SD Increase) and Size at Birth by Maternal BMI*

Maternal BMI According to WHO Classification	N	Peripheral Blood Pressures (mmHg)		Central Blood Pressures (mmHg)	
		SBP (1 SD = 11.1 mmHg) ß (95% CI)	DBP (1 SD = 8.3 mmHg) ß (95% CI)	SBP (1 SD = 10.0 mmHg) ß (95% CI)	PP (1 SD = 6.5 mmHg) ß (95% CI)
Weight (g)					
BMI <25.0 kg/m^2	329	−74.50 (−117.92 to −31.08)	−38.79 (−80.33 to 2.74)	−81.36 (−122.36 to −40.32)	−66.81 (−105.84 to −27.80)
BMI 25.0-29.9 kg/m^2	259	−26.64 (−79.84 to 26.56)	−60.87 (−111.65 to −10.10)	−41.90 (−94.50 to 10.69)	13.56 (−31.22 to 58.35)
BMI ≥30.0 kg/m^2	125	14.59 (−72.21 to 101.40)	80.71 (−8.57 to 170.01)	41.23 (−45.56 to 128.03)	−24.39 (−99.09 to 50.31)
P for interaction		0.06	0.02	0.02	0.02
Length (cm)**					
BMI <25.0 kg/m^2	327	−0.40 (−0.65 to −0.15)	−0.18 (−0.42 to 0.05)	−0.44 (−0.68 to −0.21)	−0.40 (−0.63 to −0.18)
BMI 25.0-29.9 kg/m^2	259	0.04 (−0.25 to 0.33)	−0.29 (−0.56 to −0.01)	−0.09 (−0.38 to 0.19)	0.18 (−0.06 to 0.42)
BMI ≥30.0 kg/m^2	125	−0.07 (−0.48 to 0.34)	0.51 (0.09 to 0.93)	0.12 (−0.29 to 0.54)	−0.31 (−0.66 to 0.04)
P for interaction		0.04	0.009	0.03	0.001
Head circumference (cm)**					
BMI <25.0 kg/m^2	327	−0.19 (−0.35 to −0.04)	−0.20 (−0.35 to −0.06)	−0.22 (−0.37 to −0.07)	−0.07 (−0.21 to 0.07)
BMI 25.0-29.9 kg/m^2	259	−0.08 (−0.27 to 0.10)	−0.10 (−0.28 to 0.08)	−0.12 (−0.31 to 0.06)	−0.03 (−0.19 to 0.12)
BMI ≥30.0 kg/m^2	125	0.17 (−0.11 to 0.45)	0.31 (0.02 to 0.59)	0.13 (−0.15 to 0.42)	−0.12 (−0.36 to 0.12)
P for interaction		0.04	0.004	0.05	0.87
Placenta weight (g)***					
BMI <25.0 kg/m^2	323	−17.72 (−32.74 to −2.69)	−11.45 (−25.67 to 2.76)	−20.54 (−34.79 to −6.29)	−14.05 (−27.27 to −0.83)
BMI 25.0-29.9 kg/m^2	256	−5.85 (−21.75 to 10.06)	−9.62 (−24.95 to 5.71)	−10.14 (−25.91 to 5.62)	−1.76 (−15.11 to 11.58)
BMI ≥30.0 kg/m^2	120	−0.26 (−27.15 to 26.63)	4.16 (−23.54 to 31.87)	1.23 (−25.54 to 27.99)	−2.27 (−25.05 to 20.51)
P for interaction		0.18	0.49	0.15	0.25

SD, standard deviation; CI, confidence interval; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

* Multiple linear regression models were used with adjustment for baby's sex, gestation at delivery, maternal age, ethnicity, education, parity, smoking history, height, BMI at 27 weeks gestation, coffee consumption and depression.

** There were 2 women with missing information on length and head circumference.

*** There were 14 women with missing information on placental weight.

To further account for the influence from gestational age, we used gestational age adjusted SD scores of size at birth as the outcomes, and similar findings were observed (Supplemental Tables 5-4 – 5-6). Using binary variables of birth weight, we found that higher peripheral and central blood pressures were associated with higher odds for low birth weight and small for gestational age infants (Table 5-6). Tests for interactions between maternal ethnicity and blood pressures were not significant (data not shown), whereas the interactions between maternal BMI category and blood pressures (peripheral SBP, central SBP and PP) were borderline significant for small for gestational age ($p = 0.04$ to 0.06), and the interaction between maternal BMI category and central PP was significant for low birth weight ($p = 0.01$). Again, the odds ratios were generally stronger in normal weight women compared to overweight/obese women (Table 5-6).

Table 5-6. The Association between Maternal Blood Pressures (Per 1-SD Increase) and Low Birth Weight and Small for Gestational Age

		Peripheral Blood Pressures (mmHg)		Central Blood Pressures (mmHg)	
	N	SBP (1 SD = 11.1 mmHg) OR (95% CI)	DBP (1 SD = 8.3 mmHg) OR (95% CI)	SBP (1 SD = 10.0 mmHg) OR (95% CI)	PP (1 SD = 6.5 mmHg) OR (95% CI)
Low birth weight					
All women*	713	1.64 (1.12 to 2.41)	1.82 (1.27 to 2.61)	1.85 (1.29 to 2.67)	1.17 (0.85 to 1.62)
BMI categories**					
BMI <25.0 kg/m^2	329	2.12 (1.27 to 3.53)	1.70 (1.06 to 2.72)	2.41 (1.45 to 3.99)	1.82 (1.15 to 2.88)
BMI ≥25.0 kg/m^2	384	1.09 (0.60 to 1.99)	1.88 (1.09 to 3.24)	1.28 (0.73 to 2.24)	0.62 (0.34 to 1.13)
P for interaction		0.11	0.86	0.14	0.01
Small for gestational age					
All women*	713	1.58 (1.16 to 2.14)	1.41 (1.06 to 1.89)	1.70 (1.27 to 2.28)	1.36 (1.05 to 1.76)
BMI categories**					
BMI <25.0 kg/m^2	329	1.88 (1.24 to 2.86)	1.43 (0.97 to 2.09)	2.01 (1.35 to 3.00)	1.74 (1.19 to 2.53)
BMI ≥25.0 kg/m^2	384	1.17 (0.72 to 1.92)	1.27 (0.79 to 2.03)	1.26 (0.79 to 2.03)	1.04 (0.69 to 1.55)
P for interaction		0.04	0.44	0.06	0.04

SD, standard deviation; OR, odds ratio; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

* Multiple logistic regression models were used with adjustment for baby's sex, gestation at delivery, maternal age, ethnicity, education, parity, smoking history, height, BMI at 27 weeks gestation, coffee consumption and depression. In the analysis of small for gestational age, gestation weeks at delivery was not adjusted for.

** Adjusted baby's sex, gestation at delivery, maternal age, ethnicity, education, parity, smoking history, height, BMI at 27 weeks gestation, coffee consumption and depression. In the analysis for small for gestational age, gestation at delivery was not adjusted for. Women with BMI 25.0-29.9 or ≥ 30.0 kg/m^2 were grouped together due to small sizes and problems with model convergence.

5.4 Discussion

We found associations between higher maternal blood pressures and smaller offspring. Maternal adiposity modified the associations with stronger inverse associations in normal weight women than their overweight/obese counterparts. No significant effect modification by ethnicity were found, although Chinese women with higher blood pressures tended to have smaller offspring.

Our finding of an inverse association between maternal blood pressures and offspring size at birth is consistent with previous studies.[9, 150, 152-155, 159-163] For example, Bakker et al.[9] reported that per one-SD increase in SBP and DBP at mean gestation of 30.2 weeks (range 28.4 to 32.9 weeks) was associated with 16.9 g and 50.6 g lower birth weight, respectively. Among non-hypertensive women, higher peripheral blood pressures (range of gestation 26 to 39 weeks) were also associated with lower birth weight;[155] and higher central blood pressures (range of gestation 22 to 39 weeks) were associated with lower birth weight.[159, 160]

However, there are studies with conflicting results. For example, two previous studies measuring DBP from 34 weeks gestation onwards [158] and the average of SBP and DBP during pregnancy[156] have described a u-shaped association with birth weight. In another perinatal cohort study, DBP measured between 15 to 24 weeks gestation were not found to be significantly associated with birth weight.[157] These studies[156, 158] were based on retrospective cohort design that utilized blood pressure information collected under clinical context whereas studies that reported inverse associations were prospective cohort design with blood pressure information collected by the

study investigators. Furthermore, the DBP measures reported in these retrospective cohort studies [157, 158] were based on either Korotkoff phase IV or V from standard mercury sphygmomanometer compared to the automated oscillometric device used in other studies [9, 152] or Spacelabs blood pressure monitor.[153, 154] Varying DBP measures arising from the different Korotkoff phases[59] and blood pressure devices could have contributed to the conflicting results.

Some studies have suggested that central blood pressures may be more relevant to size at birth than the conventional peripheral blood pressures, because blood pressure differences were more pronounced in central than peripheral measures.[159, 160] However, in the current analysis, we found similar effect estimates between central and peripheral blood pressures, which is consistent with an earlier report by Elvan-Taspinar et al.[155] Although central blood pressures may be better markers for arterial stiffness,[30, 62] the role of central and peripheral blood pressures in relation to offspring birth size have yet to be ascertained due to the limited and divergent literature.

The exact mechanisms linking higher maternal blood pressures and smaller offspring birth weight are unclear. Several studies have observed that women with preeclampsia and low birth weight offspring share a common link in placental dysfunction.[167-170] But whether placental dysfunction precedes maternal hypertension, or that it arises from maternal hypertension as a consequence of pre-existing maternal predisposition to endothelial dysfunction, current literature is still controversial.[152, 167, 168, 170] Although the exact mechanism is unclear, higher maternal blood pressures could be a feature shared

by both endothelial dysfunction and placental dysfunction, as both entities are not mutually exclusive.[169]

A previous study by Lydakis et al.[161] found that the relation between higher maternal blood pressures and lower birth weight was stronger in Asian Indians than white or black women, but no studies have yet tested the ethnic differences within Asian women. Our study is the first in its kind in three Asian ethnicities, and we observed no significant ethnic differences in the association between blood pressures and birth outcomes. Our results of inverse associations were also supported by some studies in Asian women, where pre-eclampsia was associated with increased risk to small for gestational age in Chinese women[162] and lower birth weight in Indian women.[163] However, we cannot exclude the possibility of ethnic differences due to the smaller subgroups of Indian and Malay women in our cohort and therefore was not powered to detect effect modification by maternal ethnicity. Our exploratory analysis on the ethnic differences in the relations between blood pressures and birth size were among the first few in literature and future studies are still needed to further explore the potential ethnic differences.

Our finding on the effect modification by maternal obesity is consistent with literature that lean or normal weight women with higher blood pressures have smaller offspring compared to their obese counterparts.[152] The effect modification by maternal obesity on fetal growth restriction, may be due to the higher fetal nutrient supply in obese women,[171] and the overall effect of maternal obesity and blood pressures on birth weight may be dependent on the balance of these factors.[152, 170]

There are several strengths of our study. The prospective design enabled the evaluation of a comprehensive information on offspring size at birth and a wide range of potential confounding factors. Peripheral blood pressure and radial pulse wave were measured in a detailed and standardized approach, thereby minimizing inter-rater measurement errors. Various sensitivity analyses suggested that our results were robust.

We are aware of several limitations. First, we excluded 38.6% of the GUSTO participants due to missing information on the exposures and covariates. However, we deemed that the selection bias was unlikely to change our results based on the sensitivity analysis using imputed data (Supplemental Table 5-2). Second, we did not have data on first and third trimester blood pressure, and thus were unable to assess trimester specific blood pressure changes during pregnancy in relation to size at birth. Thirdly, the use of maternal BMI at 27 weeks gestation may be affected by misclassification due to the growing fetus and fluid accumulation. We chose to use mid-pregnancy BMI instead of pre-pregnancy BMI because the latter measure was self-reported and thus susceptible to information bias, and about 5% of the women did not report their pre-pregnancy weights. Due to the lack of pregnancy-specific classification for obesity, we have used the WHO cut-offs for non-pregnant adults in our study. However, our sensitivity analysis of using tertiles of BMI suggested that the interaction with BMI categories was robust. We did not measure maternal weight before delivery, and could not know whether the relation between blood pressures and birth size outcomes would be changed if total weight gain during pregnancy was adjusted in the model. Our results may also be affected by residual confounding from coffee intake as it was self-

reported, and unmeasured confounding factors, like diet and physical activity, are possible to explain our results. Lastly, the non-significant maternal blood pressures and offspring birth weight relations among the Malay and Indian populations in the study may be due to the smaller numbers of women in these ethnic subgroups

In conclusion, our results provide further evidence that higher second trimester blood pressures are associated with smaller offspring, with a stronger association among normal weight women. Therefore, routine antenatal monitoring of maternal blood pressures are clinically relevant and important practice, and may have a positive impact on offspring size at birth, particularly in normal weight women.

Supplemental Table 5-1. Maternal Characteristics by Study Inclusion to the Present Study

Maternal Characteristics	Included in study		Excluded from study		*P**
	n	Mean (SD) or %	n	Mean (SD) or %	
Peripheral systolic blood pressure (mmHg)	713	109.3 (11.1)	116	110.6 (11.7)	0.25
Peripheral diastolic blood pressure (mmHg)	713	66.7 (8.2)	116	67.4 (9.9)	0.38
Central systolic blood pressure (mmHg)	713	96.7 (9.9)	116	97.9 (10.9)	0.20
Central pulse pressure (mmHg)	713	30.0 (6.5)	116	30.6 (6.8)	0.41
Age at booking (years)					0.18
1st quartile (18-26)	162	22.7%	119	27.7%	
2nd quartile (27-29)	148	20.8%	83	19.4%	
3rd quartile (30-33)	204	28.6%	104	24.2%	
4th quartile (34-46)	199	27.9%	123	28.7%	
Race					0.23
Chinese	399	55.9%	222	51.8%	
Indian	118	16.6%	87	20.3%	
Malay	196	27.5%	120	27.9%	
Education					0.37
Primary-Secondary	229	32.1%	138	32.8%	
Post-Secondary	245	34.4%	158	37.5%	
Tertiary	239	33.5%	125	29.7%	
Smoking Status					0.47
Never smoker	618	86.7%	290	85.0%	
Ever smoker	95	13.3%	51	15.0%	
Coffee Consumption					0.42
No	369	51.8%	186	54.4%	
Yes	344	48.2%	156	45.6%	
Parity					0.84
Nulliparous	311	43.6%	147	41.8%	
Primiparous	246	34.5%	124	35.2%	
Multiparous	156	21.9%	81	23.0%	
Gestational Diabetes					0.27
No	540	81.4%	304	84.2%	
Yes	123	18.6%	57	15.8%	
Pre-pregnancy BMI (kg/m^2)					0.58
<25.0	518	76.4%	243	73.4%	
25.0-29.9	113	16.7%	62	18.7%	
≥30.0	47	6.9%	26	7.8%	
2nd trimester BMI (kg/m^2)					0.19
<25.0	329	46.1%	180	49.2%	
25.0-29.9	259	36.3%	113	30.9%	
≥30.0	125	17.5%	73	19.9%	
Rate of weight gain at 27 weeks (kg/week)					0.46
1st tertile (-0.42 – 0.25)	227	33.5%	107	33.0%	
2nd tertile (0.26 – 0.37)	218	32.2%	116	35.8%	
3rd tertile (0.38 – 1.38)	233	34.4%	101	31.2%	

Maternal Depression					0.16
Not depressed	631	88.5%	300	85.5%	
Depressed	82	11.5%	51	14.5%	
Gestation at delivery (weeks)	713	38.8 (1.4)	352	38.6 (1.8)	0.006
Size at birth					
Weight (g)	713	3113.5 (435.0)	350	3034.2 (478.2)	0.007
Length (cm)	711	48.7 (2.2)	351	48.2 (2.4)	0.001
Head circumference (cm)	711	33.4 (1.36)	350	33.2 (1.6)	0.006
Placenta weight (g)	699	585.3 (118.9)	346	561.5 (124.0)	0.003

BMI, body mass index.

Data are mean (standard deviation) or % as specified.

*Using Student *t* test for continuous variables or χ^2 test for categorical variables.

Supplemental Table 5-2. Sensitivity Analysis for Per 1-SD Increase in Maternal Blood Pressures and Size at Birth

	N	Peripheral Blood Pressures (mmHg)		Central Blood Pressures (mmHg)	
		SBP (1 SD = 11.1 mmHg) ß (95% CI)	DBP (1 SD = 8.3 mmHg) ß (95% CI)	SBP (1 SD = 10.0 mmHg) ß (95% CI)	PP (1 SD = 6.5 mmHg) ß (95% CI)
Weight (g)					
Main Model	713	-35.56 (-66.57 to -4.54)	-25.13 (-55.36 to 5.09)	-40.52 (-70.66 to -10.37)	-24.10 (-51.24 to 3.03)
Main Model + GDM*	663	-34.47 (-66.93 to -1.99)	-32.77 (-64.51 to -1.03)	-41.72 (-73.05 to -10.38)	-18.99 (-47.71 to 9.71)
Main Model among non-hypertensive women	705	-36.25 (-68.02 to -4.47)	-30.16 (-61.28 to 0.97)	-42.63 (-73.65 to -11.61)	-20.83 (-48.31 to 6.64)
Main Model using multiple imputation	922	-34.00 (-65.25 to -2.75)	-24.37 (-53.15 to 4.41)	-38.43 (-67.47 to -9.39)	-21.09 (-48.67 to 6.49)
Main Model with pre-pregnancy BMI & rate of weight gain	678	-35.05 (-66.66 to -3.43)	-21.19 (-51.69 to 9.33)	-40.14 (-70.79 to -9.49)	-27.21 (-54.67 to 0.25)
Length (cm)**					
Main Model	711	-0.16 (-0.32 to 0.01)	-0.10 (-0.27 to 0.06)	-0.19 (-0.36 to -0.03)	-0.14 (-0.28 to 0.01)
Main Model + GDM*	661	-0.17 (-0.35 to 0.00)	-0.14 (-0.31 to 0.04)	-0.21 (-0.38 to -0.04)	-0.13 (-0.28 to 0.03)
Main Model among non-hypertensive women	703	-0.18 (-0.36 to -0.01)	-0.14 (-0.31 to 0.02)	-0.23 (-0.40 to -0.06)	-0.13 (-0.28 to 0.02)
Main Model using multiple imputation	921	-0.16 (-0.32 to 0.01)	-0.11 (-0.27 to 0.05)	-0.21 (-0.37 to -0.06)	-0.12 (-0.26 to 0.02)
Main Model with pre-pregnancy BMI & rate of weight gain	676	-0.15 (-0.32 to 0.02)	-0.10 (-0.27 to 0.06)	-0.19 (-0.36 to -0.02)	-0.12 (-0.28 to 0.02)
Head circumference (cm)**					

Main Model	711	-0.09 (-0.19 to 0.02)	-0.08 (-0.18 to 0.02)	-0.12 (-0.23 to -0.02)	-0.07 (-0.16 to 0.02)
Main Model + GDM*	661	-0.07 (-0.19 to 0.04)	-0.07 (-0.18 to 0.04)	-0.10 (-0.21 to 0.01)	-0.05 (-0.15 to 0.05)
Main Model among non-hypertensive women	703	-0.10 (-0.21 to 0.01)	-0.10 (-0.21 to 0.01)	-0.14 (-0.25 to -0.04)	-0.07 (-0.17 to 0.02)
Main Model using multiple imputation	921	-0.08 (-0.20 to 0.03)	-0.08 (-0.18 to 0.03)	-0.12 (-0.22 to -0.01)	-0.06 (-0.15 to 0.04)
Main Model with pre-pregnancy BMI & rate of weight gain	676	-0.09 (-0.20 to 0.02)	-0.08 (-0.18 to 0.03)	-0.12 (-0.23 to -0.02)	-0.07 (-0.17 to 0.02)
Placenta weight (g)* **					
Main Model	699	-8.78 (-18.74 to 1.19)	-6.94 (-16.63 to 2.76)	-11.16 (-20.85 to -1.47)	-6.44 (-15.04 to 2.16)
Main Model + GDM*	650	-11.48 (-22.05 to -0.92)	-9.19 (-19.51 to 1.13)	-13.27 (-23.46 to -3.07)	-7.12 (-16.32 to 2.08)
Main Model among non-hypertensive women	691	-8.93 (-19.17 to 1.31)	-7.70 (-17.72 to 2.32)	-11.54 (-21.54 to -1.54)	-5.92 (-14.65 to 2.80)
Main Model using multiple imputation	908	-8.04 (-18.21 to 2.13)	-8.23 (-17.39 to 0.93)	-11.23 (-21.31 to 1.14)	-5.64 (-13.92 to 2.65)
Main Model with pre-pregnancy BMI & rate of weight gain	666	-11.15 (-21.45 to -0.85)	-7.79 (17.70 to 2.12)	-13.01 (-22.99 to -3.02)	-7.59 (-16.43 to 1.24)

SD, standard deviation; CI, confidence interval; GDM, gestational diabetes; IOM, Institute of Medicine; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

* There were 50 women with missing information on gestational diabetes

** There were 2 women with missing information on length and head circumference.

*** There were 14 women with missing information on placental weight.

Main model was derived from multiple linear regression with adjustment for baby's sex, gestation at delivery, maternal age, ethnicity, education, parity, smoking history, maternal BMI at 27 weeks gestation, height, coffee consumption and depression.

Main Model + GDM: adjusted for covariates in Main Model with additional adjustment for gestational diabetes.

Main Model among non-hypertensive women: the analysis was done in women with peripheral systolic blood pressures less than 140 mmHg and diastolic blood pressures less than 90 mmHg.

Main Model using multiple imputation: missing data in blood pressures were imputed from model with maternal age, ethnicity, education, parity, smoking history, BMI at 27 weeks gestation, height, gestational diabetes and the respective outcome variable.

Main Model with pre-pregnancy BMI is adjusted for the same covariates in Main Model except for maternal BMI at 27 weeks gestation which was replaced by pre-pregnancy BMI and rate of weight gain per week.

Supplemental Table 5-3. Per 1-SD Increase in Maternal Blood Pressures and Size at Birth by Maternal BMI in Tertiles*

Maternal BMI in Tertiles	N	Peripheral Blood Pressures (mmHg)		Central Blood Pressures (mmHg)	
		SBP (1 SD = 11.1 mmHg)	DBP (1 SD = 8.3 mmHg)	SBP (1 SD = 10.0 mmHg)	PP (1 SD = 6.5 mmHg)
		ß (95% CI)	ß (95% CI)	ß (95% CI)	ß (95% CI)
Weight (g)					
1st Tertile	234	-89.57 (-142.41 to -36.73)	-32.09 (-82.41 to 18.23)	-95.6 (-145.66 to -45.54)	-84.53 (-129.73 to -39.33)
2nd Tertile	244	-25.05 (-77.64 to 27.53)	-53.05 (-103.05 to -3.05)	-26.05 (-76.72 to 24.62)	24.38 (-21.83 to 70.59)
3rd Tertile	235	2.21 (-57.05 to 61.47)	14.56 (-44.92 to 74.04)	-6.28 (-65.48 to 52.92)	-21.13 (-72.20 to 29.93)
P for interaction		0.03	0.09	0.03	0.008
Length (cm)**					
1st Tertile	233	-0.54 (-0.85 to -0.22)	-0.14 (-0.45 to 0.16)	-0.58 (-0.88 to -0.28)	-0.57 (-0.84 to -0.30)
2nd Tertile	243	0.08 (-0.21 to 0.37)	-0.27 (-0.54 to 0.00)	-0.01 (-0.29 to 0.26)	0.28 (0.03 to 0.53)
3rd Tertile	235	-0.03 (-0.33 to 0.27)	0.18 (-0.12 to 0.48)	0.00 (-0.30 to 0.30)	-0.17 (-0.43 to 0.09)
P for interaction		0.02	0.06	0.006	0.0001
Head circumference (cm)**					
1st Tertile	233	-0.31 (-0.49 to -0.12)	-0.22 (-0.40 to -0.05)	-0.30 (-0.48 to -0.12)	-0.13 (-0.29 to 0.03)
2nd Tertile	243	-0.06 (-0.24 to 0.13)	-0.12 (-0.30 to 0.06)	-0.10 (-0.28 to 0.08)	0.01 (-0.16 to 0.17)
3rd Tertile	235	0.08 (-0.12 to 0.28)	0.11 (-0.09 to 0.31)	0.01 (-0.19 to 0.21)	-0.10 (-0.27 to 0.08)
P for interaction		0.005	0.009	0.02	0.64
Placenta weight (g)***					
1st Tertile	230	-28.89 (-47.19 to -10.59)	-16.57 (-33.81 to 0.66)	-29.41 (-46.89 to -11.93)	-17.04 (-32.46 to -1.63)
2nd Tertile	240	6.60 (-9.09 to 22.29)	-6.38 (-21.45 to 8.68)	0.12 (-15.04 to 15.28)	7.06 (-6.71 to 20.82)
3rd Tertile	229	-8.07 (-26.95 to 10.81)	-1.07 (-19.94 to 17.81)	-9.83 (-28.60 to 8.95)	-10.18 (-26.30 to 5.94)
P for interaction		0.02	0.22	0.04	0.08

SD, standard deviation; CI, confidence interval; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

* Multiple linear regression models were used with adjustment for baby's sex, gestation at delivery, maternal age, ethnicity, education, parity, smoking history, height, BMI at 27 weeks gestation, coffee consumption and depression.

** There were 2 women with missing information on length and head circumference.

*** There were 14 women with missing information on placental weight.

Supplemental Table 5-4. Per 1-SD Increase in Maternal Blood Pressures and Gestational Age Adjusted SD Scores of Birth Size Outcomes*

Measures of Size at Birth	N	Peripheral Blood Pressures (mmHg)		Central Blood Pressures (mmHg)	
		SBP (1 SD = 11.1 mmHg) ß (95% CI)	DBP (1 SD = 8.3 mmHg) ß (95% CI)	SBP (1 SD = 10.0 mmHg) ß (95% CI)	PP (1 SD = 6.5 mmHg) ß (95% CI)
Weight (SD)	713	-0.08 (-0.17 to 0.00)	-0.06 (-0.14 to 0.02)	-0.10 (-0.18 to -0.02)	-0.06 (-0.13 to 0.01)
Length (SD)**	711	-0.07 (-0.15 to 0.02)	-0.05 (-0.13 to 0.04)	-0.08 (-0.17 to -0.003)	-0.06 (-0.14 to 0.02)
Head circumference (SD)**	711	-0.06 (-0.14 to 0.02)	-0.05 (-0.13 to 0.03)	-0.09 (-0.17 to -0.01)	-0.06 (-0.13 to 0.01)
Placental weight (SD)***	699	-0.07 (-0.16 to 0.01)	-0.06 (-0.14 to 0.02)	-0.09 (-0.18 to -0.01)	-0.05 (-0.12 to 0.02)

SD, standard deviation; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

* Measures of size at birth were estimated as gestational age adjusted standard deviation scores. Multiple linear regression models were used with adjustment for baby's sex, maternal age, ethnicity, education, parity, smoking history, height, BMI at 27 weeks gestation, coffee consumption and depression.

** There were 2 women with missing information on length and head circumference.

*** There were 14 women with missing information on placental weight.

Supplemental Table 5-5. Per 1-SD Increase in Maternal Blood Pressures and Gestational Age Adjusted SD Scores of Birth Size Outcomes by Maternal Ethnicity*

Maternal Ethnicity	N	Peripheral Blood Pressures (mmHg)		Central Blood Pressures (mmHg)	
		SBP (1 SD = 11.1 mmHg) ß (95% CI)	DBP (1 SD = 8.3 mmHg) ß (95% CI)	SBP (1 SD = 10.0 mmHg) ß (95% CI)	PP (1 SD = 6.5 mmHg) ß (95% CI)
Weight (SD)					
Chinese	399	-0.12 (-0.23 to -0.01)	-0.10 (-0.21 to 0.001)	-0.14 (-0.24 to -0.03)	-0.07 (-0.17 to 0.03)
Indian	118	0.01 (-0.20 to 0.22)	0.02 (-0.18 to 0.22)	-0.05 (-0.26 to 0.16)	-0.06 (-0.23 to 0.09)
Malay	196	-0.02 (-0.19 to 0.15)	-0.04 (-0.21 to 0.13)	-0.05 (-0.22 to 0.12)	-0.02 (-0.18 to 0.14)
P for interaction		0.90	0.80	0.96	0.98
Length (SD)**					
Chinese	398	-0.04 (-0.16 to 0.07)	-0.07 (-0.19 to 0.04)	-0.07 (-0.19 to 0.04)	-0.02 (-0.12 to 0.09)
Indian	117	-0.03 (-0.23 to 0.16)	-0.04 (-0.22 to 0.15)	-0.08 (-0.27 to 0.11)	-0.05 (-0.19 to 0.11)
Malay	196	-0.13 (-0.29 to 0.03)	0.03 (-0.13 to 0.19)	-0.11 (-0.27 to 0.05)	-0.18 (-0.33 to -0.03)
P for interaction		0.12	0.95	0.31	0.09
Head circumference (SD)**					
Chinese	398	-0.10 (-0.21 to 0.01)	-0.10 (-0.21 to 0.004)	-0.13 (-0.24 to -0.03)	-0.06 (-0.16 to 0.04)
Indian	117	-0.01 (-0.21 to 0.19)	0.09 (-0.10 to 0.28)	-0.06 (-0.26 to 0.13)	-0.14 (-0.29 to 0.02)
Malay	196	0.02 (-0.14 to 0.19)	-0.04 (-0.20 to 0.13)	0.01 (-0.16 to 0.17)	0.05 (-0.10 to 0.21)
P for interaction		0.71	0.76	0.61	0.24
Placenta weight (SD)***					
Chinese	392	-0.07 (-0.19 to 0.05)	-0.06 (-0.17 to 0.05)	-0.10 (-0.21 to 0.02)	-0.06 (-0.16 to 0.04)
Indian	115	-0.07 (-0.28 to 0.13)	-0.03 (-0.23 to 0.16)	-0.09 (-0.29 to 0.10)	-0.06 (-0.22 to 0.09)
Malay	192	-0.09 (-0.26 to 0.07)	-0.11 (-0.27 to 0.05)	-0.11 (-0.27 to 0.04)	-0.02 (-0.17 to 0.12)
P for interaction		0.84	0.89	0.88	0.99

SD, standard deviation; CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

* Measures of size at birth were estimated as gestational age adjusted standard deviation scores. Multiple linear regression models were used with adjustment for baby's sex, maternal age, ethnicity, education, parity, smoking history, height, BMI at 27 weeks gestation, coffee consumption and depression

** There were 2 women with missing information on length and head circumference

*** There were 14 women with missing information on placental weight

Supplemental Table 5-6. Per 1-SD Increase in Maternal Blood Pressures and Gestational Age Adjusted SD Scores of Birth Size Outcomes by Maternal BMI According to WHO Classification*

Maternal BMI According to WHO Classification	N	Peripheral Blood Pressures (mmHg)		Central Blood Pressures (mmHg)	
		SBP (1 SD = 11.1 mmHg) ß (95% CI)	DBP (1 SD = 8.3 mmHg) ß (95% CI)	SBP (1 SD = 10.0 mmHg) ß (95% CI)	PP (1 SD = 6.5 mmHg) ß (95% CI)
Weight (SD)					
BMI <25.0 kg/m^2	329	-0.19 (-0.31 to -0.08)	-0.09 (-0.21 to 0.01)	-0.21 (-0.32 to -0.10)	-0.18 (-0.28 to -0.08)
BMI 25.0-29.9 kg/m^2	259	-0.06 (-0.19 to 0.09)	-0.17 (-0.30 to -0.03)	-0.11 (-0.24 to 0.04)	0.05 (-0.07 to 0.17)
BMI ≥30.0 kg/m^2	125	0.05 (-0.18 to 0.29)	0.24 (-0.00 to 0.48)	0.13 (-0.10 to 0.37)	-0.06 (-0.26 to 0.14)
P for interaction		0.05	0.01	0.02	0.009
Length (SD)**					
BMI <25.0 kg/m^2	327	-0.18 (-0.31 to -0.06)	0.08 (-0.19 to 0.04)	-0.21 (-0.33 to -0.09)	-0.19 (-0.31 to -0.08)
BMI 25.0-29.9 kg/m^2	259	0.02 (-0.12 to 0.17)	-0.15 (-0.29 to -0.01)	-0.04 (-0.18 to 0.11)	0.11 (-0.01 to 0.23)
BMI ≥30.0 kg/m^2	125	-0.02 (-0.23 to 0.49)	0.28 (0.06 to 0.49)	0.08 (-0.14 to 0.29)	-0.16 (-0.33 to 0.02)
P for interaction		0.04	0.005	0.03	<0.001
Head circumference (SD)**					
BMI <25.0 kg/m^2	327	-0.15 (-0.326 to -0.03)	-0.15 (-0.26 to -0.04)	-0.18 (-0.29 to -0.06)	-0.06 (-0.17 to 0.04)
BMI 25.0-29.9 kg/m^2	259	-0.05 (-0.19 to 0.09)	-0.06 (-0.19 to 0.08)	-0.08 (-0.22 to 0.06)	-0.03 (-0.15 to 0.09)
BMI ≥30.0 kg/m^2	125	0.14 (-0.07 to 0.36)	0.26 (0.04 to 0.48)	0.13 (-0.09 to 0.34)	-0.08 (-0.27 to 0.10)
P for interaction		0.04	0.002	0.03	0.88
Placenta weight (SD)***					
BMI <25.0 kg/m^2	323	-0.16 (-0.28 to -0.03)	-0.10 (-0.23 to 0.02)	-0.18 (-0.30 to -0.06)	-0.12 (-0.24 to -0.01)
BMI 25.0-29.9 kg/m^2	256	-0.03 (-0.17 to 0.09)	-0.08 (-0.21 to 0.05)	-0.08 (-0.21 to 0.05)	-0.01 (-0.12 to 0.10)
BMI ≥30.0 kg/m^2	120	0.01 (-0.21 ti 0.24)	0.07 (-0.17 to 0.30)	0.04 (-0.19 to 0.26)	-0.02 (-0.21 to 0.18)
P for interaction		0.09	0.31	0.07	0.19

SD, standard deviation; CI, confidence interval; BMI, body mass index; WHO, World Health Organization.

* Measures of size at birth were estimated as gestational age adjusted standard deviation scores. Multiple linear regression models were used with adjustment for baby's sex, maternal age, ethnicity, education, parity, smoking history, height, BMI at 27 weeks gestation, coffee consumption and depression.

** There were 2 women with missing information on length and head circumference.

*** There were 14 women with missing information on placental weight.

CHAPTER 6

MATERNAL BLOOD PRESSURES DURING PREGNANCY AND OFFSPRING BLOOD PRESSURES AT 3 YEARS OLD

This manuscript is under review now.

Wai-Yee Lim, Yung-Seng Lee, Fabian Kok-Peng Yap, Izzudin Mohd Aris, Lek Ngee, Michael Meaney, Peter D Gluckman, Keith M Godfrey, Kenneth Kwek, Yap-Seng Chong, Seang-Mei Saw & An Pan. Maternal blood pressures during pregnancy and early childhood blood pressures in the offspring: The GUSTO birth cohort study.

6.1 Introduction

Studies have consistently showed that in-utero exposure to preeclampsia is associated with higher blood pressures and an increased risk to hypertension and cardiovascular complications later in life.[172-190] For example, a meta-analyses of observational studies has reported a 2 mmHg and 1 mmHg higher systolic (SBP) and diastolic (DBP) blood pressures, respectively, in the offspring exposed to maternal preeclampsia.[183, 184] This inter-generational transmission of higher levels of blood pressure may indicate shared mechanisms which include genetic [82] or non-genetic factors such as shared environmental or lifestyle factors.[74]

However, most of the literature on maternal-offspring blood pressures is based on maternal hypertension which included preeclampsia or gestational hypertension,[172, 176, 177, 179, 180, 182, 185-189] and few have examined the peripheral and central components and quantitative nature of blood pressures (Table 6-1).[190] Moreover, examining maternal hypertension based on prevailing blood pressure cut-offs may be suboptimal as blood pressure is a biological variable that has a unimodal distribution in the population.[191] The effects from the stable and pulsatile hemodynamic blood pressure components, which include SBP, DBP, and pulse pressure (PP) during pregnancy, has also rarely been studied. Lastly, as hypertension is increasingly prevalent in Asian populations,[71, 72] examining the influence of maternal blood pressures on early offspring blood pressures may lead to the early preventive strategies and reduce the disease burden in later life.

Therefore in the present study, we aimed to examine the relation between maternal blood pressures during pregnancy and offspring blood pressures during early childhood in a prospective mother-offspring cohort of Southeast Asian Chinese, Malay and Indian subjects.

Table 6-1. Evidence Table for the Associations Between Maternal Hypertension and Offspring Blood Pressures

ID	Author/Year	Design	Sample Size/Country	Offspring age at Blood Pressure Measurements	Measures of Blood Pressures	Results
1	Fraser et al; 2013	Prospective cohort	ALSPAC cohort of 2888 offspring with complete information on BP information/ United Kingdom	15 to 17 years old	PE or GH	SBP and DBP were higher in offspring of mother with GH or PE than those without. <u>GH</u> SBP: 2.06 (95%CI 1.28-2.84)mmHg DBP: 1.11 (95%CI 0.54-1.69)mmHg <u>PE</u> SBP: 1.12 (95%CI -0.80 -3.12)mmHg DBP: 1.71 (95%CI 0.23-3.17)mmHg
2	Miettola et al/2013	Prospective cohort	5573/Finland	16 years old	PE GH	Normal pregnancy: GH : PE SBP: 114 vs 119 mmHg ($p<0.001$) vs 116 mmHg ($p=0.15$) DBP: 67 vs 70 mmHg ($p<0.001$) vs 68 mmHg ($p=0.06$)
3	Lazdam et al/ 2012	Prospective cohort	109/ United Kingdom	Mean age 24 years old	Preterm PE and non PE	Preterm offspring with hypertension compared to normotensive pregnancies : - Have lower pulse wave velocity - Have lower flow mediated dilation - Have higher common carotid intima thickness Preterm PE: Preterm Normal: Term normal Peripheral SBP: 120.2 (11.8):12.7 (10.4): 114.0(11.0) mmHg

						Peripheral DBP: 71.8 (7.4):71.6 (6.5): 66.1 (7.0) mmHg Peripheral PP: 48.4(10.6): 48.9 (8.5): 47.9 (8.2) mmHg Central SBP: 115.9 (10.9): 115.8 (10.6): 96.7 (9.9) mmHg Central DBP: 69.4 (6.3): 68.5 (9.8): 66 (7.4) mmHg Central PP: 46.6 (8.6): 46.9 (10.3): 30.7 (4.8) mmHg
4	Geelhoed et al/ 2010	Prospective cohort	ALSPAC cohort of 6668 mother-offspring information on offspring BP/ United Kingdom	9 years old	PE or GH	SBP and DBP were higher in offspring of mother with GH or PE than those without. <u>GH</u> SBP: 2.04 (95%CI 1.42-2.67) mmHg DBP: 1.07 (95%CI 0.60-1.54) mmHg <u>PE</u> SBP: 2.05 (95%CI 0.72-3.38) mmHg DBP: 1.00 (95%CI -0.01-2.10) mmHg
5	Kvehaugen et al/ 2010	Prospective cohort	63/Norway	5-8 years old	PE	Control vs PE (median) SBP: 115 vs 119 mmHg (p=0.03) DBP: 70 vs 70 mmHg (p=0.17)
6	Oglaend et al/ 2009	Prospective cohort	537/ Norway	10.8 to 11.8 years old	PE	Crude SBP were significantly different between offspring of women with preeclampsia and normal pregnancies 113.5 vs 115.3 mmHg (diff 1.8 [0.2-3.5]mmHg) However, association were no longer significant after adjusting for maternal BMI and SBP Offspring DBP not significant different between women with preeclampsia and normal pregnancies

7	Tenhola et al/ 2006	Prospective cohort	57/Finland	12 years of age	PE	Daytime SBP; PE: non-PE (122.0 vs 108.3 mmHg, p<0.001) Nightime SBP; (117.8 vs 105.9 mmHg, p<0.001_ Daytime DBP:73.5 vs 59.1 mmHg, p<0.001) Nightime DBP: (69.7 vs 56.6 mmHg, p<0.001) Subgroup analysis between preterm and term PE – no significant difference
8	Tenhola et al/ 2003	Prospective cohort	120/Finland	12 years of age	PE	SBP; PE: Non PE (116.4 vs 113.2 mmHg, p=0.02) DBP; 73.9 vs 70.3 mmHg, p=0.02) Highest SBP and DBP in SGA PE offspring (SBP=120 mmHg; DBP=76.3 mmHg)
9	Kajantie et al/ 2009	Prospective cohort	13345/Finland	Mean age at follow-up 30 (SD 2.9) for 28.8 (SD8.8) years	PE or GH	Stroke: GH: HR 1.4 (95%CI 1.0-1.8) PE; HR 1.9 (95%CI 1.2-3.0) Offspring hypertension: GH: RR 1.3 (95%CI 1.1-1.5) Non-severe PE: RR 0.9 (95%CI 0.6-1.5) Severe PE: RR1.5 (95%CI 1.1-2.3)
10	Palti et al/ 1989	Prospective cohort	188/Israel	6 years old	PE	PE : non PE SBP; 101.3 vs 99.8 mmHg, NS DBP; 66.2 vs 63.9 mmHg, p=0.03
11	Seidman et al/1991	Prospective cohort	33545/Israel	17 years	PE	High SBP; Female: OR 2.3 (95%CI 1.80 – 4.46) Not significant for SBP in males; DBP for all

12	Vik et al/ 2013/ Norway	Cross-sectional study/ Data Linkage	36528 father-mother and offspring trios with complete BP, weight and height information were analysed (linking record from the 3 HUNT Surveys)	Offpsring age 35.6 (10.6)	Maternal SBP and DBP	Parent offspring associations of BP are similar between fathers and mothers. Maternal-offspring correlation for SBP is stronger than paternal-offspring (-0.21 95%CI -0.04, -0.004; p=0.02) No difference for DBP

PE, preeclampsia; GH, gestational hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure

6.2 Methods

Study Population

The **G**rowing **U**p in **S**ingapore **T**owards healthy **O**utcomes (GUSTO) study is a prospective mother-offspring cohort study where 1162 pregnant women less than 14 weeks gestation were recruited at two public tertiary hospitals with maternity care in Singapore from 2009 to 2010.[192] Women who were enrolled into the GUSTO study were free of type 1 diabetes and were not on chemotherapy treatment or on psychotropic drugs. From the GUSTO study cohort, 829 women had mid-pregnancy blood pressure measurements and were eligible for the present study. Among the offspring of these women, 567 had blood pressure measurements at age 3 years and these 567 maternal-offspring pairs with complete blood pressure information were included in the present study (Figure 6-1). The women included in the analysis tended to be older, had higher education and were less likely to smoke and consume alcohol compared to the 262 women excluded. Maternal blood pressures during pregnancy and offspring blood pressures at 3 years old were similar between women who were included and excluded in the analysis (Supplemental Table 6-1). The study was approved by the SingHealth Centralised Institutional Review Board and National Healthcare Group Domain Specific Review Board. Written informed consent was obtained from the study participants.

Figure 6-1. Flow Chart of the GUSTO Study Sample Selected for Analysis

Blood pressure Measurements

Based on a standardized protocol, maternal blood pressures were taken by trained research coordinators during the GUSTO mid-pregnancy follow-up visits at a median gestation of 27 weeks (interquartile range 26 to 29 weeks).

Mothers were rested for at least 10 minutes prior to blood pressure measurement, and the peripheral SBP and DBP were measured thrice from the brachial arm at 30-60 second intervals with an oscillometric device MC3100 (HealthSTATS International Pte Ltd, Singapore). An average of these three readings was calculated if the difference between readings was less than 10 mmHg; otherwise, measurements were repeated. Central blood pressures were determined by the radial artery pressure waveforms measured from the A-pulse tonometer (BPro®, HealthSTATS International Pte Ltd, Singapore), having calibrated with the average of peripheral SBP and DBP, respectively. Central SBP were then estimated from the calibrated radial artery pressure waveforms using the N-point moving average.[12] Peripheral and central pulse pressure (PP) was calculated as the difference between peripheral or central SBP and peripheral DBP.

Offspring Blood Pressure Outcomes

At the age of 3 years old, offspring blood pressure outcomes were measured by trained research personnel at the outpatient clinics. Prior to blood pressure taking, the child was required to seat with the mother for at least 5 minutes in a quiet room. Peripheral SBPs and DBPs were taken twice from the right brachial arm using a Dynamap CARESCAPE™ V100 (GE Healthcare, Milwaukee, WI, USA) with the arm resting at the chest level. An average of the two blood pressure readings was calculated if the difference between readings were less than 10 mmHg; otherwise, a third reading was taken and an average of the 3 readings was taken instead.

Covariates

Information on maternal age, ethnicity and education level, smoking status, alcohol and coffee consumption and physical activity during pregnancy, family history of hypertension, number of living children and pre-pregnancy weight were obtained via questionnaires and maternal height was measured by trained research coordinators at GUSTO mid-pregnancy follow-up. Maternal pre-pregnancy BMI was calculated as weight (kg) divided by the square of height (m^2). Information on the offspring's sex and gestation at birth were retrieved from medical records and their subsequent weight and height at 3 years old were measured at the same time of their blood pressure measurements.

Statistical Analysis

Comparisons between characteristics of eligible mother-offspring pairs that were included and excluded from the current analysis were done using Student's t-test and Chi-square test for continuous and categorical variables, respectively. Partial correlations between maternal and offspring blood pressures were performed using Pearson correlations, adjusted for offspring sex and ethnicity. Associations between continuous variables of maternal blood pressures (peripheral SBP, DBP, PP, central SBP and PP) and offspring blood pressures (peripheral SBP, DBP and PP) were examined using multiple linear regression with adjustments for maternal age, education level, parity, smoking status, alcohol consumption and physical activity during pregnancy, and pre-pregnancy BMI; offspring characteristics (including sex, ethnicity, BMI and height at 3 years of age) were also included. As there were only 30

women with hypertension during pregnancy (17 with gestational hypertension, 9 with preeclampsia and 4 with chronic hypertension), we did not include maternal hypertension as a covariate in our analysis. Instead, we repeated our analysis in women without hypertension in pregnancy (n=537).

We further evaluated the relation of binary variables of maternal blood pressures with offspring blood pressures. The continuous variables of maternal blood pressures were categorized into binary variables with 85th percentile higher maternal blood pressures based on >120 mmHg for peripheral SBP as prehypertension,[60] >75 mmHg for peripheral DBP, >50 mmHg for peripheral PP, >106 mmHg for central SBP and >35 mmHg for central PP.

Exploratory analysis on effect modifications by offspring sex (male, female) and ethnicity (Chinese, Malay, or Indian) were evaluated from the multiplicative interaction terms between continuous blood pressure variables and the effect modifier added to the main effect model. Ethnicity and sex stratified analysis was performed, respectively, and likelihood ratio test was used to test for interaction effects. All analysis were performed using Stata version 11.2 (Statacorp, College Station, Texas) was used for analysis; and two tailed P value of less than 0.05 was considered statistically significant.

6.3 Results

Of the 567 mother-offspring pairs followed-up in the present study, 310 (54.7%) were of Chinese, 157 (27.7%) Malay and 100 (17.6%) Indian ethnicity. The mean age of women enrolled was 31.0 (SD 5.1) years. Overall,

higher maternal blood pressures were observed in women of Malay ethnicity, lower education, who smoked before, had no or light physical activity during pregnancy, or higher pre-pregnancy BMI (Table 6-2). Maternal blood pressures also tended to be higher in hypertensive women, but offspring blood pressures were similar in the children of hypertensive and normotensive women (Table 6-3).

Table 6-2.Distribution of Maternal Blood Pressures by Maternal and Offspring's Characteristics*

Characteristics	Overall	Peripheral Blood Pressures (mmHg)					Central Blood Pressures (mmHg)			
		SBP	*P*	DBP	*P*	PP	*P*	SBP	*P*	PP	*P*
Age, years			0.71		0.26		0.28		0.35		0.35
1st quartile (18–26)	106 (18.7%)	110.2 ± 11.6		66.3 ± 8.4		43.9 ± 8.5		96.7 ± 10.1		30.4 ± 6.6	
2nd quartile (27–29)	132 (23.3%)	109.6 ± 10.7		67.2 ± 8.5		42.3 ± 7.8		96.6 ± 10.0		29.4 ± 6.1	
3rd quartile (30–33)	151 (26.6%)	108.6 ± 10.9		65.8 ± 8.1		42.8 ± 8.2		96.4 ± 10.1		30.5 ± 6.8	
4th quartile (34–46)	178 (31.4%)	109.5 ± 11.2		67.4 ± 8.2		42.0 ± 8.2		98.1 ± 10.1		30.7 ± 6.6	
Race			<0.001		0.003		0.09		<0.001		0.33
Chinese	310 (54.7%)	110.9 ± 11.1		65.9 ± 8.1		42.1 ± 8.2		95.9 ± 10.0		29.9 ± 6.5	
Malay	157 (27.7%)	112.8 ± 11.2		69.0 ± 8.4		43.8 ± 7.8		99.8 ± 10.2		30.8 ± 6.1	
Indian	100 (17.6%)	108.4 ± 10.3		65.6 ± 8.2		42.7 ± 8.6		96.2 ± 9.3		30.6 ± 7.5	
Education			0.006		0.001		0.80		0.002		0.72
Primary to Secondary	171 (30.3%)	110.2 ± 11.1		67.5 ± 8.4		42.7 ± 7.5		98.0 ± 10.0		30.5 ± 6.1	
GCE/Vocational/ Polytechnic	194 (34.4%)	110.8 ± 11.7		67.9 ± 8.3		42.9 ± 8.9		98.3 ± 10.5		30.3 ± 6.9	
Tertiary and above	199 (35.3%)	107.5 ± 10.3		65.1 ± 8.0		42.3 ± 8.1		95.1 ± 9.5		29.9 ± 6.6	
Alcohol intake			0.92		0.29		0.36		0.61		0.04
None	372 (67.6%)	109.2 ± 11.0		66.3 ± 8.2		42.8 ± 8.2		97.0 ± 10.2		30.6 ± 6.7	
Yes	178 (32.4%)	109.3 ± 11.2		67.1 ± 8.4		42.2 ± 8.4		96.5 ± 9.8		29.4 ± 6.3	
Smoking status			0.02		0.10		0.11		0.04		0.29
Non-smoker	497 (88.0%)	109.0 ± 10.9		66.5 ± 8.2		42.4 ± 8.3		96.7 ± 9.8		30.1 ± 6.6	
Ever-smoker	68 (12.0%)	112.4 ± 12.0		68.2 ± 9.0		44.2 ± 7.4		99.3 ± 11.6		31.1 ± 6.5	
Parity			0.77		0.81		0.87		0.33		0.24
Nulliparous	234 (41.3%)	109.3 ± 11.4		66.6 ± 7.9		42.6 ± 8.6		96.5 ± 10.0		29.9 ± 6.8	
Multiparous	333 (58.7%)	109.5 ± 10.8		66.8 ± 8.6		42.7 ± 7.9		97.4 ± 10.1		30.5 ± 6.4	
Physical activity			0.21		0.02		0.55		0.01		0.42
None to Light	404 (71.2%)	109.8 ± 11.1		67.2 ± 8.3		42.5 ± 8.1		97.7 ± 10.2		30.4 ± 6.7	
Moderate to strenuous	163 (28.8%)	108.5 ± 10.9		65.5 ± 8.2		43.0 ± 8.4		95.4 ± 9.7		29.9 ± 6.3	

Pre-pregnancy BMI, kg/m^2			<0.001		<0.001		0.02	<0.001		0.39
BMI <25.0	387 (74.1%)	107.0 ± 10.3		65.0 ± 7.5		42.0 ± 8.0		94.9 ± 9.5	29.9 ± 6.5	
BMI 25.0-29.9	93 (17.8%)	113.9 ± 9.8		70.9 ± 8.6		42.9 ± 7.9		101.4 ± 8.9	30.5 ± 6.1	
BMI ≥30.0	42 (8.05%)	119.9 ± 10.7		74.1 ± 7.8		45.7 ± 9.3		105.3 ± 9.1	31.2 ± 6.7	
Offspring Sex			0.13		0.03		0.85	0.27		0.27
Male	437 (53.8%)	108.7 ± 11.2		66.0 ± 8.0		42.7 ± 8.2		96.6 ± 10.0	30.5 ± 6.3	
Female	375 (46.2%)	110.2 ± 10.9		67.5 ± 8.6		42.6 ± 8.2		97.5 ± 10.1	29.9 ± 6.8	

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure

Data are presented as column percentages (%) or in mean ± SD

A total of 567 women and their offspring were included in analysis, women with missing information for education (n=3), alcohol intake (17), smoking status (n=2) and maternal pre-pregnancy BMI (n=45) were coded as missing.

Table 6-3. Maternal and Offspring Blood Pressures by Maternal Hypertension

	Overall (n=567)	Maternal Hypertension*		
		Non-Hypertension (n= 537)	Hypertension (n=30)	*P*
Maternal Measures				
Peripheral SBP	109.5 ± 11.1	108.8 ± 10.8	120.7 ± 10.9	<0.001
Peripheral DBP	66. ± 8 8.3	66.3 ± 8.1	75.4 ±7.5	<0.001
Peripheral PP	42.7 ± 8.2	42.6 ± 8.1	45.3 ± 10.0	0.08
Central SBP	97.1 ± 10.1	96.5 ± 9.8	107.7 ± 9.5	<0.001
Central PP	30.3 ± 6.6	30.2 ± 6.6	32.3 ± 6.3	0.08
Offspring Measures				
Peripheral SBP	98.4 ± 9.8	98.3 ± 9.9	99.6 ± 8.9	0.49
Peripheral DBP	58.3 ± 6.6	58.3 ± 6.6	58.9 ± 6.6	0.65
Peripheral PP	40.0 ± 7.0	40.0 ± 7.0	40.7 ± 7.1	0.60

SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

P values were derived from Student's t test.

*Maternal hypertension included women with chronic or gestational hypertension or preeclampsia

After accounting for offspring sex and ethnicity, maternal blood pressures were weakly correlated with offspring peripheral SBP and PP (adjusted r ranged from 0.05 to 0.11), but not with offspring peripheral DBP. Weak correlations were also observed between offspring blood pressures, BMI and height (r ranged from 0.11 to 0.25; Supplemental Table 6-2).

In the multiple linear regression models, positive maternal-offspring blood pressure relations were observed for the blood pressure measures of SBP and PP (Table 6-4) but not for DBP. Each 1-mmHg increase in maternal central SBP was associated with 0.08 (95% confidence interval [CI] 0.00 to 0.17; p=0.06) mmHg higher SBP in the offspring. Similarly, each 1-mmHg increase in maternal central

PP was associated with 0.10 (95% CI 0.01 to 0.18; p=0.03) mmHg higher offspring PP. All other maternal-offspring blood pressure relations were not statistically significant, except for the relations between maternal SBP and offspring PP. Estimated increases in offspring peripheral SBP and PP were consistently greater for maternal central blood pressures than for the relations with maternal peripheral blood pressures. Findings were not significantly modified by offspring ethnicity and sex (Supplemental Tables 6-3 and 6-4).

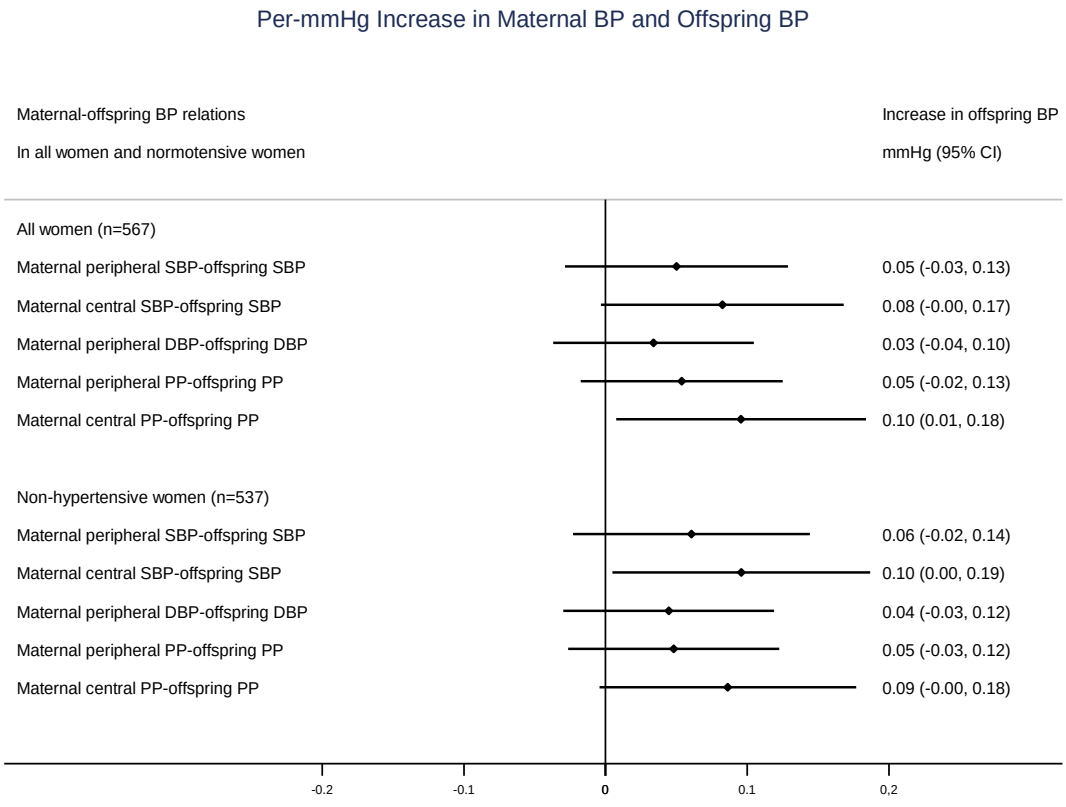
Table 6-4. Estimated Increases in Offspring Peripheral Blood Pressures, Each 1-mmHg Increase in Maternal Blood Pressures*

Maternal Blood Pressures (mmHg)	Offspring Blood Pressure Outcomes (mmHg)					
	Peripheral SBP		Peripheral DBP		Peripheral PP	
	ß (95% CI)	*P*	ß (95% CI)	*P*	ß (95% CI)	*P*
Peripheral SBP	0.05 (-0.03 to 0.13)	0.21	-0.02 (-0.07 to 0.04)	0.52	0.07 (0.01 to 0.13)	0.02
Peripheral DBP	0.09 (-0.01 to 0.20)	0.09	0.03 (-0.04 to 0.10)	0.35	0.06 (-0.02 to 0.13)	0.14
Peripheral PP	0.00 (-0.10 to 0.09)	0.96	-0.06 (-0.12 to 0.01)	0.09	0.05 (-0.02 to 0.13)	0.14
Central SBP	0.08 (0.00 to 0.17)	0.06	0.00 (-0.06 to 0.05)	0.88	0.09 (0.02 to 0.15)	0.006
Central PP	0.04 (-0.08 to 0.16)	0.50	-0.05 (-0.14 to 0.03)	0.19	0.10 (0.01 to 0.18)	0.03

CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

*A total of 567 women and their offspring were included in analysis.

All analysis were performed using multiple linear regressions with adjustments for maternal age, education level, parity, smoking status, alcohol consumption and physical activity during pregnancy, pre-pregnancy BMI; and offspring sex, ethnicity, BMI and height at 3 years of age.

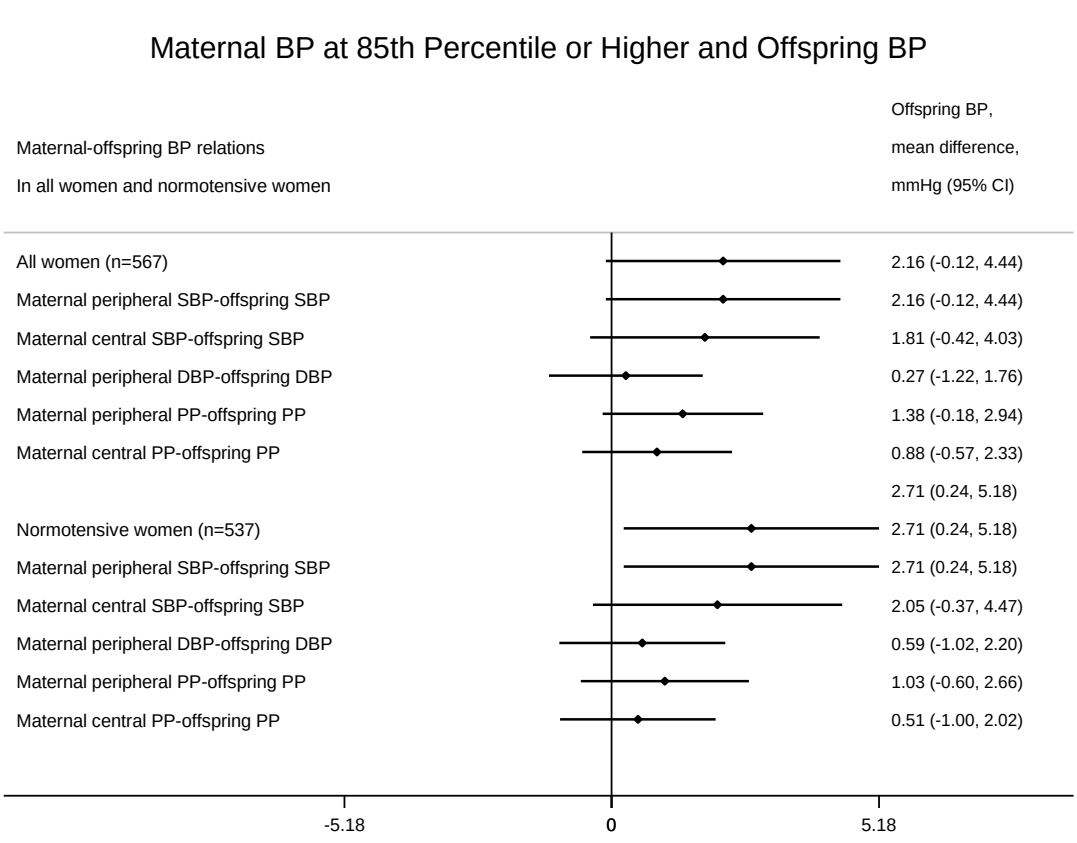


BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure

Figure 6-2. Each 1-mmHg increase in Maternal Blood Pressures at 26 – 28 Weeks Gestation and Offspring Blood Pressures

Among the 567 women included in the analysis, 537 (94.7%) were normotensive during pregnancy and only 30 (5.3%) were hypertensive during pregnancy. The maternal-offspring blood pressure relations in the subgroup of women who were normotensive during pregnancy were qualitatively the same with the main cohort (Figure 6-2; Supplemental Table 6-5). Further analysis of binary variables of maternal blood pressure $\geq 85^{th}$ percentile showed similar positive associations with offspring blood pressure (Figure 6-3; Supplemental Table 6-6). For example, weak trends were observed between offspring SBP and

maternal peripheral and central SBP at ≥85th percentile, at a mean differences of 2.16 mmHg and 1.81 mmHg respectively.



BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure

Figure 6-3. Maternal blood pressures at 85th percentile or higher at 26 – 28 Weeks Gestation and Offspring Blood Pressures

6.4 Discussion

Previous studies have shown higher blood pressures in young children and adult, who are offspring of women with pregnancy-related hypertension than offspring of women with normal pregnancy.[172-184] The present study builds on that

knowledge by demonstrating that higher maternal central blood pressures in pregnancy were associated with higher pulsatile blood pressure components (SBP and PP) in the offspring. Importantly, the positive associations of mother-offspring blood pressures persisted in normotensive women who were free of hypertension during pregnancy.

Positive maternal-offspring blood pressure relations have been reported previously in several studies that measured peripheral blood pressures[190, 193] and maternal hypertension in non-pregnant[194, 195] and pregnant women.[172-184] In the HUNT Study of Norwegians, positive correlations were reported between mother-offspring SBP (r=0.15) and DBP (r=0.14).[190] In a study of Dutch families with children aged 5-19 years, each mmHg increase in maternal peripheral SBP was associated with a 0.09 mmHg increase in offspring SBP; for DBP relation the corresponding mother-offspring increase was 0.04 mmHg.[193] Compared to these findings, we found qualitatively similar maternal-offspring blood pressure correlations in our cohort of Southeast Asian Chinese, Malay and Indian women. In our study, each 1mmHg increase in maternal peripheral and central SBP was associated with 0.05 mmHg and 0.08 mmHg increases in offspring SBP, although the former was not statistically significant.

Our observation of stronger associations of maternal central than peripheral blood pressures with offspring blood pressures is supported by two studies that suggest central blood pressures may be better markers for arterial compliance than peripheral blood pressures.[30, 196] Although two recent meta-analysis have confirmed the predictive value of central blood pressures for cardiovascular risk

and outcomes in pregnant[197] and non-pregnant [198] populations, evidence for its stronger role compared with peripheral blood pressures was demonstrated only in non-pregnant populations.[64] Therefore, further studies examining maternal blood pressures during pregnancy incorporating both central and peripheral measures are needed.

In line with the present literature on the positive associations between pregnancy hypertension and offspring blood pressures,[172-184] our study extend these findings by suggesting that maternal blood pressures during pregnancy are an important determinant of offspring blood pressures, even in normotensive pregnant women. Indirect evidence came from studies examining the influence of maternal blood pressures in normotensive pregnancies, whereby higher maternal blood pressures have been found to be associated with smaller offspring[9, 10] and preterm delivery.[10] Therefore, it is plausible that maternal blood pressures, even within normal range, have a graded relation with offspring blood pressures.

The positive association of mother and offspring blood pressures, as early as 3 years old in the present cohort, suggests that the higher blood pressures in offspring of women with higher pregnancy blood pressures occur early in life. And, as blood pressure tracks in life,[199] the higher blood pressure in the offspring of women with higher pregnancy blood pressures are also likely to persist in life. This has been demonstrated in studies that examined maternal hypertension in pregnant[172-184] and non-pregnant women[194, 195] in relation to blood pressures in offspring aged between 5 to 30 years of age.

The higher early childhood SBP and PP in offspring of women with higher blood pressures are likely to persist through adulthood and may have significant impact on cardiovascular health in later life. SBP and PP, being indicators of arterial stiffening,[200, 201] are associated with increased risk of cardiovascular morbidity and mortality,[201] While this raises the possibility that optimal blood pressure compliance through antenatal monitoring and primary health prevention strategies could have long term benefits for offspring health this hypothesis can only be addressed in randomized trials.

Overall, the results in the present study are congruent with earlier studies that the blood pressure phenotype clusters in family, and this may be explained by the shared environmental,[74, 202] epigenetic and/or genetic factors.[203-205] Moreover, findings from animal and in-utero studies have found changes in the cardiac and vascular structures [204, 205] as well as sympatho-adrenal and renal dysfunction in the offspring exposed to maternal hypertension during pregnancy.[203]

The findings of this study should be interpreted in consideration of its strengths and limitations. Strengths of this study include the prospectively measured maternal and offspring blood pressures, performed according to a standard protocol by trained research personnel and the ability to measure and account for various maternal and offspring factors in our analysis. However, there are several limitations to our study. First, 262 (31.4%) mother-offspring pairs were excluded from the present analysis due to missing offspring blood pressure information at the GUSTO 3rd year follow-up. However, our findings are unlikely to be affected by selection bias as the information on maternal blood pressures

was similar between those who were included and excluded from analysis. As maternal blood pressures were measured only once during pregnancy, we were unable to examine the blood pressure changes during pregnancy in relation to blood pressures in the offspring. Our effect estimates may be affected by residual confounding, for example, from self-reported measures of maternal pre-pregnancy BMI and from lack of adjustment for dietary intake (like salt).[74, 206, 207] As salt intake is an important determinant for offspring blood pressures, this may have limited the interpretation of our study findings. However, the maternal-offspring blood pressure relations are likely to persist as findings from the Avon Longitudinal Study of Parents and Children had demonstrated persistent positive maternal hypertension and offspring blood pressure relations even after adjustment for salt intake.[176] Measuring blood pressures in young children at the age of 3 years old have practical difficulties, for example having the child seated comfortably and taking repeated measurements in the same position, and therefore, greater variation and measurement errors are likely to occur. Lastly, the non-significant effect modification by ethnicity may be constrained by the smaller ethnic subgroups of Chinese, Malay and Indian women. Further studies in Asian women are needed to explore their ethnic contributions to the maternal-offspring blood pressure relations.

In conclusion, our findings suggest that higher maternal blood pressures during pregnancy are associated with higher offspring blood pressures. As the maternal-offspring blood pressure relations were observed even in normotensive

pregnant women, ensuring optimal blood pressure compliance during pregnancy has long term implications in the cardiovascular health in the offspring.

Supplemental Table 6-1. Characteristics of Women and Their Offspring, Comparing Those Included and Excluded From Analysis

Characteristics	Overall	Women Included	Women Excluded	*P*
n	829 (100%)	567 (68.4%)	262 (31.6%)	
Maternal Characteristics				
Age, years	30.4 ± 5.2	31.0 ± 5.1	29.1 ± 5.03	<0.001
Race				0.53
Chinese	451 (54.4%)	310 (54.7%)	141 (53.8%)	
Malay	238 (28.7%)	157 (27.7%)	81 (30.9%)	
Indian	140 (16.9%)	100 (17.6%)	40 (15.3%)	
Education				0.01
Primary to Secondary	267 (32.6%)	171 (30.3%)	96 (37.5%)	
GCE/Vocational/Polytechnic	289 (35.2%)	194 (34.4%)	95 (37.1%)	
Tertiary and above	264 (32.2%)	199 (35.3%)	65 (25.4%)	
Alcohol intake				0.03
None	526 (65.2%)	372 (67.6%)	154 (59.9%)	
Yes	281 (34.8%)	178 (32.4%)	103 (40.1%)	
Smoking status				0.03
Non-smoker	710 (86.2%)	497 (88.0%)	213 (82.2%)	
Ever-smoker	114 (13.8%)	68 (12.0%)	46 (17.8%)	
Parity				0.11
Nulliparous	350 (43.1%)	234 (41.3%)	116 (47.4%)	
Multiparous	462 (56.9%)	333 (58.7%)	129 (52.6%)	
Physical activity				0.02
None to Light	609 (73.7%)	404 (71.2%)	205 (79.2%)	
Moderate to Strenuous	217 (26.3%)	163 (28.8%)	54 (20.8%)	
Maternal Hypertension				0.11
None	787 (94.9%)	537 (94.7%)	250 (95.4%)	
Chronic Hypertension	5 (0.4%)	4 (0.7%)	1 (0.2%)	
Gestational Hypertension	25 (2.2%)	17 (3.0%)	8 (1.3%)	
Preeclampsia	21 (1.8%)	9 (1.6%)	12 (2.0%)	
Pre-pregnancy BMI, kg/m^2	22.8 ± 4.4	22.8 ± 4.4	22.6 ± 4.4	0.59
Peripheral SBP, mmHg	109.49 ± 11.16	109.47 ± 11.12	109.53 ±11.27	0.94
Peripheral DBP, mmHg	66.78 ±8.46	66.77 ± 8.35	66.80 ± 8.73	0.95
Peripheral PP, mmHg	42.71 ±8.27	42.70 ± 8.24	42.73 ± 8.34	0.97
Central SBP, mmHg	96.89 ± 10.09	97.06 ± 10.10	96.5 ± 10.06	0.45
Central PP, mmHg	30.11 ± 6.54	30.29 ± 6.59	29.69 ± 6.42	0.22
Offspring Characteristics				
Sex				0.21
Male	437 (53.8%)	297 (52.4%)	140 (57.1%)	
Female	375 (46.2%)	270 (47.6%)	105 (42.9%)	
BMI, kg/m^2	15.8 ± 1.6	15.8 ± 1.6	15.9 ± 1.9	0.54
Height, cm	94.8 ± 3.8	94.8 ± 3.8	94.9 ± 4.0	0.78
Peripheral SBP, mmHg	-	98.4 ± 9.8	-	
Peripheral DBP, mmHg	-	58.3 ± 6.6	-	
Pulse pressure, mmHg	-	40.0 ± 7.0	-	

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure

Data are presented as mean ± SD or in column percentages (%)

A total of 567 women and their offspring were included in analysis, women with missing information for education (n=3), alcohol intake (17), smoking status (n=2) and maternal pre-pregnancy BMI (n=45) were coded as missing.

Supplemental Table 6-2. Partial Correlations between Maternal and Offspring's Anthropometry and Blood Pressure Measures

	Offspring Blood Pressure Outcomes (mmHg)					
	Peripheral SBP		Peripheral DBP		Peripheral PP	
	r	*P*	r	*P*	r	*P*
Maternal Measures						
Peripheral SBP, mmHg	0.07	0.08	0.01	0.78	0.09	0.03
Peripheral DBP, mmHg	0.07	0.08	0.05	0.19	0.05	0.21
Peripheral PP, mmHg	0.02	0.58	-0.04	0.34	0.07	0.09
Central SBP, mmHg	0.09	0.04	0.02	0.69	0.11	0.01
Central PP, mmHg	0.04	0.35	-0.04	0.30	0.10	0.02
Pre-pregnancy BMI, kg/m^2	0.05	0.29	0.06	0.15	0.01	0.89
Offspring Measures						
BMI at 3 years old, kg/m^2	0.25	<0.001	0.23	<0.001	0.14	<0.001
Height at 3 years old, cm	0.18	<0.001	0.11	0.007	0.14	<0.001

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

Partial correlations coefficients were adjusted for offspring sex and ethnicity.

Supplementary Table 6-3. Estimated Increases in Offspring Peripheral Blood Pressures, Each 1-mmHg Increase in Maternal Blood Pressures in Chinese, Malay and Indian women

Maternal Blood Pressures (mmHg)	Offspring Blood Pressure Outcomes (mmHg)					
	Peripheral SBP		Peripheral DBP		Peripheral PP	
	ß (95% CI)	*P*	ß (95% CI)	*P*	ß (95% CI)	*P*
Peripheral SBP						
All women	0.05 (-0.03 to 0.13)	0.21	-0.02 (-0.07 to 0.04)	0.52	0.07 (0.01 to 0.13)	0.02
Chinese	0.12 (0.02 to 0.22)	0.02	0.01 (-0.05 to 0.08)	0.72	0.11 (0.03 to 0.18)	0.005
Malay	0.01 (-0.18 to 0.20)	0.91	-0.01 (-0.12 to 0.10)	0.85	0.02 (-0.10 to 0.15)	0.73
Indian	-0.05 (-0.25 to 0.14)	0.58	-0.13 (-0.30 to 0.04)	0.14	0.07 (-0.08 to 0.23)	0.35
P for interaction	0.45		0.76		0.38	
Peripheral DBP						
All women	0.09 (-0.01 to 0.20)	0.09	0.03 (-0.04 to 0.10)	0.35	0.06 (-0.02 to 0.13)	0.14
Chinese	0.15 (0.02 to 0.28)	0.02	0.06 (-0.02 to 0.15)	0.15	0.09 (-0.01 to 0.19)	0.08
Malay	0.15 (-0.12 to 0.41)	0.27	-0.02 (-0.18 to 0.14)	0.82	0.17 (-0.01 to 0.35)	0.07
Indian	-0.07 (-0.29 to 0.16)	0.57	0.09 (-0.11 to 0.29)	0.39	-0.15 (-0.33 to 0.03)	0.09
P for interaction	0.57		0.51		0.12	
Peripheral PP						
All women	0.00 (-0.10 to 0.09)	0.96	-0.06 (-0.12 to 0.01)	0.09	0.05 (-0.02 to 0.13)	0.14
Chinese	0.05 (-0.08 to 0.17)	0.44	-0.04 (-0.12 to 0.04)	0.36	0.09 (-0.01 to 0.18)	0.07
Malay	-0.11 (-0.35 to 0.14)	0.39	0.00 (-0.15 to 0.14)	0.97	-0.10 (-0.27 to 0.06)	0.22
Indian	-0.01 (-0.23 to 0.21)	0.94	-0.25 (-0.43 to -0.06)	0.01	0.24 (0.07 to 0.41)	0.006
P for interaction	0.59		0.23		0.05	
Central SBP						
All women	0.08 (0.00 to 0.17)	0.06	0.00 (-0.06 to 0.05)	0.88	0.09 (0.02 to 0.15)	0.006
Chinese	0.14 (0.04 to 0.25)	0.006	0.02 (-0.05 to 0.09)	0.53	0.12 (0.04 to 0.20)	0.004
Malay	0.09 (-0.13 to 0.31)	0.41	0.01 (-0.12 to 0.13)	0.93	0.08 (-0.06 to 0.23)	0.25
Indian	-0.01 (-0.21 to 0.19)	0.90	-0.06 (-0.24 to 0.11)	0.48	0.05 (-0.11 to 0.21)	0.53
P for interaction	0.54		0.85		0.59	
Central PP						
All women	0.04 (-0.08 to 0.16)	0.50	-0.05 (-0.14 to 0.03)	0.19	0.10 (0.01 to 0.18)	0.03
Chinese	0.09 (-0.07 to 0.24)	0.26	-0.04 (-0.14 to 0.06)	0.44	0.13 (0.01 to 0.25)	0.03
Malay	-0.02 (-0.34 to 0.30)	0.92	0.04 (-0.15 to 0.23)	0.69	-0.05 (-0.27 to 0.16)	0.62
Indian	0.06 (-0.19 to 0.30)	0.65	-0.2 (-0.41 to 0.02)	0.07	0.25 (0.07 to 0.44)	0.009
P for interaction	0.76		0.33		0.18	

CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

All analysis were performed using multiple linear regressions with adjustments for maternal age, education level, smoking status, alcohol consumption and physical activity during pregnancy, pre-pregnancy BMI; and offspring sex, BMI and height at 3 years of age.

A total of 310 Chinese, 157 Malay and 100 Indian women and their offspring were analysed.

Supplementary Table 6-4. Estimated Increases in Offspring Peripheral Blood Pressures, Each 1-mmHg Increase in Maternal Blood Pressures in Male and Female Offspring

Maternal Blood Pressures (mmHg)	Offspring Blood Pressure Outcomes (mmHg)					
	Peripheral SBP		Peripheral DBP		Peripheral PP	
	ß (95% CI)	*P*	ß (95% CI)	*P*	ß (95% CI)	*P*
Peripheral SBP						
All offspring	0.05 (-0.03 to 0.13)	0.21	-0.02 (-0.07 to 0.04)	0.52	0.07 (0.01 to 0.13)	0.02
Males	0.03 (-0.08 to 0.14)	0.58	-0.01 (-0.08 to 0.05)	0.69	0.04 (-0.04 to 0.12)	0.28
Females	0.07 (-0.05 to 0.19)	0.26	-0.03 (-0.11 to 0.06)	0.55	0.09 (0.01 to 0.18)	0.03
P for interaction	0.74		0.76		0.47	
Peripheral DBP						
All offspring	0.09 (-0.01 to 0.20)	0.09	0.03 (-0.04 to 0.10)	0.35	0.06 (-0.02 to 0.13)	0.14
Males	0.04 (-0.11 to 0.19)	0.59	0.05 (-0.04 to 0.15)	0.27	-0.01 (-0.12 to 0.10)	0.83
Females	0.15 (0.00 to 0.30)	0.06	0.01 (-0.10 to 0.12)	0.82	0.13 (0.03 to 0.24)	0.01
P for interaction	0.56		0.46		0.15	
Peripheral PP						
All offspring	0.00 (-0.10 to 0.09)	0.96	-0.06 (-0.12 to 0.01)	0.09	0.05 (-0.02 to 0.13)	0.14
Males	0.01 (-0.12 to 0.15)	0.84	-0.07 (-0.15 to 0.02)	0.13	0.08 (-0.02 to 0.18)	0.12
Females	-0.03 (-0.17 to 0.11)	0.67	-0.05 (-0.15 to 0.05)	0.35	0.02 (-0.08 to 0.12)	0.73
P for interaction	0.78		0.73		0.48	
Central SBP						
All offspring	0.08 (0.00 to 0.17)	0.06	0.00 (-0.06 to 0.05)	0.88	0.09 (0.02 to 0.15)	0.006
Males	0.07 (-0.05 to 0.19)	0.26	0.02 (-0.05 to 0.10)	0.58	0.05 (-0.04 to 0.14)	0.30
Females	0.09 (-0.03 to 0.22)	0.16	-0.04 (-0.13 to 0.06)	0.44	0.13 (0.04 to 0.22)	0.01
P for interaction	0.94		0.29		0.28	
Central PP						
All offspring	0.04 (-0.08 to 0.16)	0.50	-0.05 (-0.14 to 0.03)	0.19	0.10 (0.01 to 0.18)	0.03
Males	0.09 (-0.08 to 0.26)	0.31	-0.03 (-0.14 to 0.08)	0.63	0.12 (-0.01 to 0.25)	0.08
Females	-0.02 (-0.19 to 0.15)	0.81	-0.08 (-0.21 to 0.04)	0.20	0.06 (-0.06 to 0.18)	0.33
P for interaction	0.43		0.54		0.61	

CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

All analysis were performed using multiple linear regressions with adjustments for maternal age, education level, smoking status, alcohol consumption and physical activity during pregnancy, pre-pregnancy BMI; and offspring ethnicity, BMI and height at 3 years of age.

A total of 297 male offspring and 270 female offspring were analysed.

Supplemental Table 6-5. Estimated Increases in Offspring Peripheral Blood Pressures, Each 1-mmHg Increase in Maternal Blood Pressures in All Women and Normotensive Women.

Maternal Blood Pressures (mmHg)	N	Offspring Blood Pressure Outcomes (mmHg)					
		Peripheral SBP		Peripheral DBP		Peripheral PP	
		ß (95% CI)	*P*	ß (95% CI)	*P*	ß (95% CI)	*P*
Peripheral SBP							
All women	567	0.05 (-0.03 to 0.13)	0.21	-0.02 (-0.07 to 0.04)	0.52	0.07 (0.01 to 0.13)	0.02
Normotensive women	537	0.06 (-0.02 to 0.14)	0.16	-0.01 (-0.06 to 0.05)	0.86	0.07 (0.01 to 0.13)	0.03
Peripheral DBP							
All women	567	0.09 (-0.01 to 0.20)	0.09	0.03 (-0.04 to 0.10)	0.35	0.06 (-0.02 to 0.13)	0.14
Normotensive women	537	0.10 (-0.01 to 0.21)	0.07	0.04 (-0.03 to 0.12)	0.24	0.06 (-0.02 to 0.14)	0.15
Peripheral PP							
All women	567	0.00 (-0.10 to 0.09)	0.96	-0.06 (-0.12 to 0.01)	0.09	0.05 (-0.02 to 0.13)	0.14
Normotensive women	537	0.00 (-0.10 to 0.10)	0.98	-0.05 (-0.12 to 0.02)	0.19	0.05 (-0.03 to 0.12)	0.21
Central SBP							
All women	567	0.08 (0.00 to 0.17)	0.06	0.00 (-0.06 to 0.05)	0.88	0.09 (0.02 to 0.15)	0.006
Normotensive women	537	0.10 (0.00 to 0.19)	0.04	0.01 (-0.05 to 0.07)	0.76	0.09 (0.02 to 0.15)	0.01
Central PP							
All women	567	0.04 (-0.08 to 0.16)	0.50	-0.05 (-0.14 to 0.03)	0.19	0.10 (0.01 to 0.18)	0.03
Normotensive women	537	0.05 (-0.08 to 0.17)	0.46	-0.04 (-0.12 to 0.05)	0.36	0.09 (0.00 to 0.18)	0.06

CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

Normotensive women referred to women without chronic or gestational hypertension or preeclampsia

All analysis were performed using multiple linear regressions with adjustments for maternal age, education level, parity, smoking status, alcohol consumption and physical activity during pregnancy, pre-pregnancy BMI; and offspring sex, ethnicity, BMI and height at 3 years of age.

Supplemental Table 6-6. Estimated Mean Difference in Offspring Peripheral Blood Pressures, Maternal Blood Pressures at 85th Percentile or Higher*, in All Women and Non-Hypertensive Women.

Maternal Blood Pressures		Offspring Blood Pressure Outcomes					
		Peripheral SBP		Peripheral DBP		Peripheral PP	
		ß (95% CI)	*P*	ß (95% CI)	*P*	ß (95% CI)	*P*
Peripheral SBP							
All women	567	2.16 (-0.12 to 4.44)	0.06	0.23 (-1.32 to 1.78)	0.77	1.93 (0.27 to 3.60)	0.02
Non-hypertensive women	537	2.71 (0.24 to 5.18)	0.03	0.54 (-1.13 to 2.21)	0.53	2.17 (0.38 to 3.96)	0.02
Peripheral DBP							
All women	567	1.51 (-0.69 to 3.70)	0.18	0.27 (-1.22 to 1.76)	0.72	1.23 (-0.38 to 2.84)	0.13
Non-hypertensive women	537	2.03 (-0.36 to 4.41)	0.10	0.59 (-1.02 to 2.20)	0.47	1.44 (-0.29 to 3.16)	0.10
Peripheral PP							
All women	567	0.95 (-1.18 to 3.09)	0.38	-0.43 (-1.87 to 1.02)	0.56	1.38 (-0.18 to 2.94)	0.08
Non-hypertensive women	537	0.85 (-1.40 to 3.10)	0.46	-0.18 (-1.70 to 1.34)	0.82	1.03 (-0.6 to 2.66)	0.22
Central SBP							
All women	567	1.81 (-0.42 to 4.03)	0.11	-0.27 (-1.78 to 1.25)	0.73	2.08 (0.45 to 3.70)	0.01
Non-hypertensive women	537	2.05 (-0.37 to 4.47)	0.10	-0.02 (-1.65 to 1.61)	0.98	2.07 (0.32 to 3.82)	0.02
Central PP							
All women	567	0.75 (-1.23 to 2.73)	0.46	-0.13 (-1.48 to 1.21)	0.85	0.88 (-0.57 to 2.33)	0.23
Non-hypertensive women	537	0.50 (-1.58 to 2.58)	0.64	-0.01 (-1.41 to 1.39)	0.99	0.51 (-1.00 to 2.02)	0.51

SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

* 85th percentile higher maternal peripheral SBP is > 120 mmHg, peripheral DBP > 75 mmHg, peripheral PP > 510 mmHg, central SBP > 106 mmHg and central PP > 35 mmHg.

Non-hypertensive women referred to women without chronic or gestational hypertension or preeclampsia

All analysis were performed using multiple linear regressions with adjustments for maternal age, education level, smoking status, alcohol consumption and physical activity during pregnancy, pre-pregnancy BMI, offspring sex, ethnicity, BMI and height at 3 years of age.

CHAPTER 7

GENERAL DISCUSSION

7.1 Introduction

Blood pressure is an independent arterial marker for hypertension and cardiovascular outcomes.[50, 60, 62-64] Data from several large observational studies have demonstrated a direct relation between higher maternal blood pressures and adverse pregnancy outcomes in the women and their offspring.[9, 10, 63, 157, 158] As the vast majority of literature examined maternal hypertension and few have comprehensively examined maternal blood pressures, the specific influences from the quantitative nature and components of blood pressures are not well elucidated.

Therefore the role of maternal lifestyle factors (obesity and n-3 PUFA) on maternal blood pressures, and the influence of maternal blood pressures on offspring outcomes were examined accordingly in this thesis. The following sections of this chapter provides a summary of findings, linking the evidence together. A general discussion on the methodological approach is also provided.

7.2 Summary of Findings

Based on a birth cohort of a Southeast Asian cohort of Chinese, Malay and Indian women residing in Singapore, the two main aims of the study on maternal life-style determinants and offspring consequences of maternal blood pressures during pregnancy were examined. The findings were summarized and presented accordingly in the following paragraphs.

Firstly, on the lifestyle factors of maternal blood pressures, a cross-sectional analysis of maternal adiposity and blood pressures measured at 26 – 28 weeks gestation, showed positive associations between maternal adiposity (measured by BMI and sum of skinfold thickness) and peripheral and central blood pressures. Effect modifications by maternal ethnicity and gestational diabetes were observed. The maternal adiposity-BP relations tended to be stronger Chinese women and women with gestational diabetes. Particularly, greater adiposity was associated with higher blood pressures among Chinese women with gestational diabetes.

Secondly, on the cross-sectional analysis of maternal plasma PC n-3 and 6 PUFA levels and blood pressures measured at 26 – 28 weeks gestation, total and long chain n-3 PUFAs and n-3/n-6 ratio were inversely associated with peripheral SBP and central SBP and PP, whereas dihomo-ɣ-linolenic acid was marginally positively associated with peripheral and central SBP. Higher n-3 PUFAs were also associated with lower odds of pregnancy associated hypertension [OR 0.76 (95% CI 0.60 to 0.97) in total n-3 PUFA and OR 0.77 (95%CI 0.60 to 0.98) in long chain n-3 PUFAs. Maternal ethnicity modified the relation between plasma PC PUFAs and blood pressures, with stronger inverse associations for n-3 PUFAs in Chinese women, and stronger but positive associations for n-6 PUFAs in Indian women.

Thirdly, on the consequences of maternal blood pressures on offspring outcomes, analysis of data from women with complete blood pressure information and offspring size at birth, showed that higher maternal blood pressures measured at 26 – 28 weeks gestation were associated with smaller

offspring at birth. Maternal adiposity modified the relation between maternal blood pressures and offspring size at birth, with stronger inverse associations in normal weight women than overweight/obese women. Although the effect modification by maternal ethnicity was not statistically significant, Chinese women with higher blood pressures tended to have smaller offspring compared to Malay or Indian women.

Lastly, analysis of data from maternal-offspring pairs with complete blood pressure information, showed that higher maternal blood pressures in pregnancy were associated with higher pulsatile components (SBP and PP) of blood pressures in the offspring, with larger estimated increases for maternal central blood pressures than peripheral blood pressures. When stratified by maternal hypertension, the positive mother-offspring blood pressures persisted in normotensive women who were free of hypertension during pregnancy. Effect modification by maternal ethnicity was not statistically significant.

7.3 Synthesis of Findings

From the studies included in this thesis, our findings suggest that maternal blood pressure is an important marker for haemodynamic adaptation during pregnancy. As pregnancy is associated with marked cardiovascular changes,[6, 29, 30] maternal lifestyle factors during pregnancy leading to higher pregnancy BMI may impair the mechanism involved in blood pressure control. And in turn, maladaptation in maternal blood pressures may lead to unfavourable in-utero environment for fetal growth and development,[75, 76] and thereby, influencing offspring health outcomes early in life.

We also postulate that there are two important incidental findings spanning across the four different studies. The first incidental finding was based on the ethnic differences observed in our study findings. Although the ethnic varying effect on blood pressure relations may have a genetic basis,[208-210] migratory studies have shown that between population blood pressure differences are strongly associated with environmental factors.[65, 211-213] This requires further study of gene-environment interaction in other settings, for example, to examine ethnic differences among south-east Asian women who live in rural areas and of various epidemiological study designs such as case-control or genetic linkage studies.

Secondly, we also postulate that maternal blood pressures, even in non-hypertensive women, are associated with health outcomes in the offspring. There is evidence suggesting that higher blood pressures in normotensive pregnant women are associated with preterm birth[9, 10] and smaller offspring.[9] This may be explained by the nature of blood pressure which has a Gaussian distribution in the population, and has a graded relation with cardiovascular outcomes.[19, 214, 215] Therefore, stratification of women into hypertensive or non-hypertensive groups may be inadequate to identify women at risk to having adverse outcomes in their offspring.

The other consideration of interest is the inclusion of central blood pressures measured using radial applanation tonometry. Although our findings do not suggest the superiority of central blood pressures over peripheral (brachial) blood pressures, there is some evidence suggesting its role over and above peripheral blood pressures.[64, 216] Majority of the studies compared

central and peripheral blood pressures indirectly, as they were strongly correlated with each other and thereby a methodological constraint in statistical analysis. [64, 217] At present, central blood pressure remains to be an arterial marker for research but not for clinical purposes.[17, 218]

7.4 Methodological Considerations

In this thesis, all the data used were drawn from the GUSTO birth cohort study and therefore, the findings should be interpreted in consideration of its methodological strengths and weaknesses. A general discussion on the methodological considerations involving study design, associations due to chance, confounding and bias would be described in the following.

Study Design

The GUSTO birth cohort study is prospective cohort study that was designed to examine early life factors that may be associated with metabolic compromise and altered body composition in the offspring. Therefore, the study measures that were incorporated into the GUSTO study were primarily observational in nature, and they were collected over the course of the GUSTO study follow-up (Chapter 2). Therefore, the four studies that were included in this thesis were similarly observational in nature.

A cross-sectional design was employed to examine the role of maternal adiposity, plasma PUFA on maternal blood pressures. The primary limitation associated with cross-sectional study is the difficulty to establish the temporal sequence between exposures (maternal adiposity and plasma PUFA) on

outcome (blood pressures) as they are simultaneously measured.[219, 220] However, it is noteworthy that the relation between maternal adiposity and blood pressures have been reported previously in other cohort studies in pregnant women.[30, 77, 88-92] Similarly for the PUFA and blood pressure relations, the hypotensive effect from n-3 PUFA supplementation has been shown in systematic reviews of randomized clinical trials in adult non-pregnant[79, 122, 123, 221, 222] and pregnant population,[147] although data in pregnant population has been limited. There is also evidence that weight loss combined with increased PUFAs intake have an addictive hypotensive effect compared to either effect alone,[223, 224] as shown in two randomized trials involving hypertensive[223] and non-hypertensive populations.[224] Therefore, although reverse causation may not be excluded, the significant associations observed between maternal adiposity, plasma PUFAs and blood pressures in this thesis are likely to be valid observations as they are consistent with reports from systematic reviews of randomized trials or prospective studies.[223, 224]

A prospective cohort design was employed to examine the role of maternal blood pressures on health outcomes on the offspring (size at birth and blood pressures at 3 years old). As cohort studies enable the establishment of the temporal sequence between the exposure and outcome, they are often regarded as the best for non-interventional epidemiological studies.[219, 220] Moreover, the prospective nature of the GUSTO cohort study has enabled the blood pressures in the women and their offspring to be measured systematically within specific study periods using a standardized protocol by trained research personnel.

Associations due to chance

By convention, the assessment of the role of chance in epidemiological studies involves statistical analysis in estimation of p value and confidence intervals. In this regard, the test for significance in all the statistical testing was set at 0.05 so as to reduce the probability of committing type I error.[219, 220] Although multiple analysis of peripheral and central blood pressure measures were performed, Bonferroni correction was not applied in the analysis as the blood pressure measures were highly correlated and that the tests were planned at the start of the study. [225, 226] Applying the Bonferroni correction, in this instance, will be counter-productive as it will lead to the problem of over-correction and thereby increasing the probability of type II error in the study findings. As there is active on-going research on central blood pressures, future reports will be useful in validating the findings presented in this thesis.

Confounding

Confounding is an inherent issue in all epidemiological studies. It occurs when the association between an exposure and outcome is distorted due to a third variable that is associated with both the exposure and outcome. [219, 220] This third variable however does not lie between the causal pathway between the exposure and outcome. For the maternal blood pressure associations, various strategies involving restriction, stratification and multivariate analysis were employed to control for confounding. Selection of confounders (maternal or offspring) were based on their biological importance or known confounding from the literature. For example, maternal age, ethnicity, BMI, were considered as biologically important covariates; whereas maternal education

and smoking status were considered as known confounders. Other known confounders that were included in the adjustment were based on literature review and they were specific to the blood pressure relations examined. For example, fish oil supplementation is an important confounder for the maternal blood pressures and plasma PUFAs relations; and maternal coffee intake and depression for the maternal blood pressure and offspring size at birth relations. Although there were some variations in the covariates selected for adjustment in the maternal blood pressure relations, for example maternal intake of alcohol and physical activity; the lack of adjustment for these variables are not likely alter the study findings as they are likely to only weaken the blood pressure associations to the null from residual confounding.

Residual confounding occur as a result of unmeasured or inaccurately measured, misclassified or poorly categorized variables such as maternal weight, physical activity and diet. Key covariates, such as maternal adiposity and salt intake,[65, 77, 202, 227] may have greater contribution to residual confounding, compared to others, due to their strong relationships with blood pressures across all ages of life.[65] Although maternal pre-pregnancy or pregnancy BMI have been widely used as an indication of adiposity during pregnancy, it is affected by measurement errors from the self-reported measures for pre-pregnancy BMI; and from fluid accumulation, fetal and placental weight which contributes to pregnancy BMI. Compared to maternal adiposity, measurement of salt intake from diet or urine is time consuming and often under-estimated due to difficulties in quantifying the sodium levels in recipes and discretionary salt in the diet.[228]

Selection and Information Bias

Selection bias may occur when subjects identified for study participation are dependent on the outcome of interest.[219] Therefore selection bias is a particular problem in case-control and retrospective cohort study designs where both study exposure and outcome have already occurred. [219, 220] But for the GUSTO study, selection bias due to non-participation is unlikely to occur as at the time of study enrolment, as the outcomes of interest have yet to be ascertained. Moreover, examination of data in women included and excluded from the 4 studies in this thesis, showed that there were no significant differences between exposure status between women who were included and excluded from the study.

However, selection bias due to loss to follow-up or attrition bias may be an issue in the examination of maternal blood pressures on offspring outcomes. Among GUSTO participants that had complete blood pressure information and were followed-up, a total of 116 (14.0%) were lost to follow-up due to missing information on the birth measures in the offspring (Chapter 5), and 262 (31.6%) due to missing blood pressure information in the offspring. Again, as the maternal measures of blood pressures were similar between women who were included in the study and those who were lost to follow-up, it is likely that the loss to follow-up occurred at random. Therefore, this may have affected the power, but not the internal validity of the study.

Another major source of bias in cohort studies arises from information bias. It relates to systematic difference in the measurement of exposure or outcome data for different subgroups of the study sample.[219] In the GUSTO

study, misclassification bias arising from inaccuracies or errors in recording or reporting exposure and outcome status are likely to be random and therefore would weaken the strength of the blood pressure associations to the null. Although random misclassification of maternal blood pressures and offspring outcomes may occur, the GUSTO study has built in strategies to reduce information bias through careful construction of the data collection form and rigorous training to its research personnel following standardized protocol. Moreover, maternal blood pressures were measured prior to offspring health outcomes, and the research personnel involved in the collection of outcome information in the offspring were unaware of the status of maternal blood pressures at the time of blood pressure measurements. Therefore, the impact of random misclassification of maternal blood pressures and offspring outcomes are likely to be minimized.

CHAPTER 8

CONCLUSION & FUTURE DIRECTIONS

8.1 Conclusion

In conclusion, findings from the studies included in this thesis that were based on the GUSTO cohort, indicated that maternal adiposity, n-3 and 6 PUFA levels are associated with maternal blood pressures at 26-28 weeks gestation. In our cohort of Southeast Asian Chinese, Malay and Indian women, associations between maternal characteristics (adiposity, gestational diabetes and n-3 PUFA levels) and blood pressures were stronger in Chinese women. In addition, our findings also suggest that higher maternal blood pressures during pregnancy are associated with smaller offspring size at birth and higher early childhood blood pressures.

Overall, the study findings included in this thesis are relevant to clinical practice and the pregnant woman. For example, a one percent increase in total n-3 PUFAs is associated with 0,5 mmHg decrease in peripheral SBP and a 23 percent reduction in odds to pregnancy hypertension; and likewise, a one mmHg increase in peripheral SBP is associated with a 35.6 g reduction in offspring weight at birth and 64 percent increase in odds to low birth weight. Although the effect estimates from quantitative assessment of maternal blood pressures, at per-mmHg, are small, they are relevant to clinical practice on two basis. First, the lower blood pressures levels during pregnancy from higher n-3 PUFAs if maintained over time, may reduce the risk to hypertension and cardiovascular diseases later on in life as women with pregnancy hypertension have a twofold increased risk to cardiovascular outcomes.[2] Second, the persistent maternal blood pressures and offspring outcomes in normotensive women, indicates the importance of antenatal blood pressures even if they

were not at hypertensive range. Therefore, at the population level, maintaining lower blood pressures during pregnancy will shift the average mean to lower levels, and this may have an impact on the population's risk to hypertension during pregnancy and later on in life.[229]

The observations on maternal blood pressures in the GUSTO cohort are in agreement with findings in the current literature indicating that maternal blood pressures during pregnancy may have long term consequences on offspring health outcomes. Therefore, preventive strategies that focus on prevention and detection of hypertension should be instituted early in pregnancy. Strategies that incorporate appropriate weight gain during pregnancy according to pre-pregnancy BMI and fish oil supplementation or fatty fish intake may be useful adjuncts to promoting good blood pressures control during pregnancy. This approach has been shown to be effective in improving metabolic syndrome features[223, 224] and reducing the risk of cardiovascular in women through the anti-inflammatory and immune regulatory effects from long chain PUFAs in fish oil.[230, 231] Due to the long term implications of maternal blood pressures during pregnancy on maternal and offspring's cardiovascular health, institution of preventive strategies early in pregnancy may be a good start for the pregnant woman and her family.

8.2 Future Directions

Hypertension in pregnancy is a multi-factorial disorder that has been shown to be associated with environmental and genetic risk factors.[20, 45, 67] Although the exact mechanism is unknown, deranged endothelial function characterized by

down-regulation of vasodilators and up-regulation of vasoconstrictors,[21, 45] has been proposed as one the key pathophysiological mechanism leading to higher maternal blood pressures. Other mechanisms involved were increased shear stress from hyperdynamic circulation, decrease in renin-angiotensin-aldosterone activities, increased cytokine-mediated oxidative stress, immune mediated altered inflammation and genetic factors.[20, 44]

While these mechanisms may explain the associations between maternal obesity, plasma PUFAs and blood pressures; how these mechanisms occur and interact at various levels of function, for example from genetic inheritance to expression is not well understood. As such, research in various 'omics' techniques may be an important complement to current epidemiological research.[232] Epigenomics research holds promise to many chronic diseases that are associated with genetic and environmental factors, such as hypertension and diabetes, as it not only provides the mechanisms underlying transcriptional regulation across developmental time-frames, but also the linkage between genotype and phenotype associations.[233]

In the GUSTO study, there is an on-going collection of offspring's bio-specimens for epigenetic markers from birth onwards. And the combination of epidemiological, genetic and epigenetic information from this cohort, and from other studies, will be useful to clarify the genetic-phenotype disease interactions.

In consideration of the methodological constraints in the studies described in this thesis, there are several areas of future research worth mentioning. First, the studies described in this thesis were based on information from

maternal blood pressures measured only once at 26 – 28 weeks gestation. Therefore, incorporation of maternal blood pressures measured repeatedly during the three pregnancy trimesters would provide two important levels of information in understanding the maternal blood pressure adaptation and status during pregnancy. They include trimester specific blood pressure information as well as the changes that occur during pregnancy.

Second, the associations between maternal adiposity and PUFA on maternal blood pressures suggest that these lifestyle factors may be important strategies in blood pressure control during pregnancy. However, as these findings were based on observational designs, they need to be tested further in randomised clinical trial design. Moreover, as adiposity and diet are closely related, concurrent assessment of these two determinants would facilitate the understanding on their independent and collective roles in blood pressure control strategies.

Third, as the observation on ethnic modifying effects on the maternal adiposity and PUFA levels in relation to blood pressures were novel findings, it requires confirmation in larger cohort studies. Future studies examining ethnic differences in the present target population of Southeast Asian Chinese, Malay and Indian women would need to ensure adequate power for interaction analysis. This could be achieved by oversampling of the Malay and Indian ethnic minorities. Collection of both genetic and dietary information would enable the assessment of gene-environment-interaction which may elucidate mechanisms underlying ethnic differences in relation to blood pressures.

Fourth, on the positive maternal-offspring blood pressure relations during pregnancy, the duration of follow-up in the offspring should be lengthened or until overt hypertension or cardiovascular disease occurs. The inclusion of parental (maternal and paternal) blood pressures to be examined to further elucidate and compare in-utero and ex-utero exposure to parental blood pressures. By examining the offspring early exposures to parental blood pressures, early targeted preventions may be instituted in the women or the family as a whole.

In summary, the proposed areas for future research may lead to improvement in antenatal care to ensuring optimal in-utero milieu for fetal growth and development and eventually lead to better health outcomes for both the women and their offspring.

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PUBLICATION LIST DURING PhD

1. **Wai-Yee Lim**, Kenneth Kwek, Yap-Seng Chong, Yung-Seng Lee, Fabian Yap, Yiong-Huak Chan, Keith M. Godfrey, Peter D. Gluckman, Seang-Mei Saw and An Pan. Maternal adiposity and blood pressure in pregnancy: varying relations by ethnicity and gestational diabetes. *J Hypertens*. 2014 Apr;32(4):857-64. doi: 10.1097/HJH.0000000000000096.

2. **Wai-Yee Lim**, Mary Chong, Philip C Calder, Kenneth Kwek, Yap-Seng Chong, Peter D Gluckman, Keith M Godfrey, Seang-Mei Saw and An Pan. Relations of plasma polyunsaturated fatty acids with blood pressures during the 26th and 28th week of gestation in women of Chinese, Malay, and Indian ethnicity. Medicine. 2015; 94(9):e571. doi: 10.1097/MD.0000000000000571.

3. **Wai-Yee Lim**, Yung-Seng Lee, Chuen-Seng Tan, Kenneth Kwek, Yap-Seng Chong, Peter D Gluckman, Keith M Godfrey, Seang-Mei Saw and An Pan. The association between maternal blood pressures and offspring size at birth in Southeast Asian women. *BMC Pregnancy Childbirth*. 2014 Dec 2;14(1):403.

Other relevant publication not included in this thesis:

Wai-Yee Lim, Seang-Mei Saw, Kok-Hian Tan, George S-H Yeo and Kenneth Kwek Y-C. A cohort evaluation on arterial stiffness and hypertensive disorders in pregnancy. *BMC Pregnancy Childbirth*. 2012 Dec 26;12:160. doi: 10.1186/1471-2393-12-160.

AWARD DURING PhD

Public Health Conference 2012, Singapore – Effect of obesity of central aortic pressure as a measure of arterial stiffness (Best Poster Award)

APPENDICES

RECRUITMENT VISIT
1ST CLINIC VISIT QUESTIONNAIRE

Study ID : _____ Date of interview : _____

Interviewer code : _____ Interview start time : _____

1. DEMOGRAPHY

I would like to start by asking you some questions about yourself.

1.1. How old were you when you left long term full time education?
(enter current age if still studying) ☐☐ years

1.2. What is the highest level of education that you have attained?

☐ 1: None
2: Primary (PSLE)
3: Secondary (GCE 'O'/ 'N' levels)
4: ITE/NTC
5: GCE 'A' levels/Polytechnic/diploma
6: University
7: Others, specify:_____

1.3. What is your marital status?

☐ 1: Single and living with the baby's father
2: Single and **not** living with the baby's father
3: Married (living with husband)
4: Married but **not** living with husband
5: Separated
6: Divorced
7: Widowed
8: Others, specify:_____

1.4. What is your religion?

☐ 1: No religion
2: Buddhism
3: Christianity
4: Islam
5: Taoism
6: Hinduism
7: Others, specify:_____

1.5. Where were you born?

☐ 1: Singapore ***go to question 2.1***
2: Malaysia
3: China
4: India
5: Others, specify:_____

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

1.6. When did you move to Singapore?

M	M		Y	Y	Y	Y

2. OCCUPATION

2.1. What is your current job?

- 1: Legislator/senior official
- 2: Professional
- 3: Technician & associated professional
- 4: Clerical worker
- 5: Service worker
- 6: Agricultural worker
- 7: Production craftsman
- 8: Plant and machine operator
- 9: Homemaker
- 10: Retired
- 11: Student
- 12: Unemployed
- 13: Others, specify:_____
- 14: Refused

3. HOUSING AND HOUSEHOLD COMPOSITION

3.1. What type of accommodation do you live in?

- 1: 1-2 room HDB flat
- 2: 3 room HDB flat
- 3: 4-5 room HDB flat
- 4: HUDC/executive flat
- 5: Condominium
- 6: Landed property
- 7: Others, specify:_____

3.2. Does anyone else live together with you?

- 0: No ***go to question 4.1***
- 1: Yes ***please specify in the following table (next page)***

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

For each person living in the household (apart from the woman herself), complete one line.
A household is defined as a group of people who share a living room or eat together for at least one meal a day.

CHILDREN

*For **all children**, record date of birth (or age if D.O.B. not available).*
*For the **woman's own children**, give the child's birth weight.*

S/N	Relationship to woman	Sex		D.O.B			Age (yrs)	Child's birth weight (Specify in gm or lb.oz)
		M	F	DD	MM	YYYY		
1								
2								
3								
4								
5								

ADULT

S/N	Relationship to woman	Sex		Age (yrs)	Smoker (Yes=1, No=0)
		M	F		
6					
7					
8					
9					
10					

4. CHILDCARE ARRANGEMENTS

4.1. Do you have your own child or children at home under the age of 12 years?

☐ 0: No, ***go to question 5***
1: Yes

4.1.1 If yes, you are:

☐ 1: Working part time, ***go to question 4.2***
2: Working full time, ***go to question 4.2***
3: Stay home mother, ***go to question 5***

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

4.2. Which of the following best describes the way you arrange for your child/children aged 12 or under to be looked after while you are at work?
Please fill in numbers of relevant choices in boxes on right. You can select up to 3 choices.

1: I work only while they are at school.
2: They look after themselves until I get home.
3: I work from home.
4: My husband/partner looks after them.
5: A nanny/grandparent/relative looks after them at home
6: They go to a workplace nursery.
7: They go to a day nursery.
8: They go to a child minder.
9: A relative looks after them.
10: A friend or neighbour looks after them.
11: Others, specify_____

1st choice ☐
2nd choice ☐ ☐ No further choices
3rd choice ☐ ☐ No further choices

5. PERSONAL HEALTH

Now, I would like to ask you about your personal health and about the stress level you face.

5.1. How is your health in general? Would you say it is:

☐ 1: Very good
2: Good
3: Fair
4: Bad
5: Very bad

5.2. Do you have any long term illness or disability? By long term, I mean anything that has troubled you over a period of time**?**

☐ 0: No ***go to question 5.4***
1: Yes

5.3. What is the illness/disability? _____

_____

(Do not record headaches, indigestion, aches and pains. We are interested in major problems such as diabetes, multiple sclerosis, rheumatoid arthritis, muscular dystrophy – anything which might affect growth or body composition.)

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

5.4. To what extent do you feel that the stress or pressure you have experience in your life has affected your health?

☐ 1: None
2: Slightly
3: Moderately
4: Quite a lot
5: Extremely

5.5. In general, how much stress or pressure have you experienced in your daily living in the last 4 weeks?

☐ 1: None
2: Just a little
3: A good bit
4: Quite a lot
5: A great deal

5.6. Were you part of a multiple birth (twins, triplets etc.)?

☐ 0: No
1: Yes

5.7. Were you born early, late or when your maternal mother was expecting you?

☐ 1: Early
2: When expected***, go to question 5.9***
3: Late
99: Don't know***, go to question 5.9***

5.8. How early/late were you?

☐☐ Wks ☐ Days ☐ 99: Don't know

5.9. How many children did your mother have before you were born? (including stillbirths)

☐☐ ☐ 99: Don't know

5.10. Approximately what was your weight before this pregnancy?

☐☐☐ kg ☐ 99: Don't know

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

6. ASTHMA

6.1. Have you ever suffered from asthma, either as a child or an adult?

☐ 0: No ***go to question 6.3***
1: Yes
99: Don't know ***go to question 6.3***

6.1.1. If yes, was this confirmed by a doctor?

☐ 0: No
1: Yes
99: Don't know

6.2. How many attacks of wheezing have you had in the last 12 months?

☐ 0: None
1: 1-3
2: 4-12
3: More than 12

6.3. Did you suffer from eczema (recurrent itchy skin) in childhood?

☐ 0: No ***go to question 6.5***
1: Yes
99: Don't know

6.4. Have you had eczema (recurrent itchy skin) affecting the creases of your elbows or knees in the last year?

☐ 0: No
1: Yes

6.5. Have you ever had a problem with sneezing, or a runny, or blocked nose when you did not have a cold or flu?

☐ 0: No ***go to question 6.7***
1: Yes
99: Don't know ***go to question 6.7***

6.5.1. If "YES", is the nose problem usually accompanied by itchy-watery eyes?

☐ 0: No
1: Yes
2: Sometimes
99: Don't know

6.6. In the last 12 months, have you had a problem with sneezing, or a runny, or blocked nose when you did not have a cold or the flu?

☐ 0: No
1: Yes

6.7. In the last 12 months, have you used any medicines to treat hay fever, rhinitis, or any other nasal problems, at any time (including sprays, solutions, pills, capsules or tablets)?

☐ 0: No
1: Yes

7. HIGH BLOOD PRESSURE (HYPERTENSION)

7.1. Has a doctor, a nurse or other healthcare professional ever told you that you have high blood pressure?

☐ 0: No ***go to question 8.1***
1: Yes

7.2. At what age were you diagnosed to have high blood pressure? *(Fill in one of the options below)*

Age ☐☐ (or) Year ☐☐☐☐ ☐ 99: Don't know

8. DIABETES MELLITUS

8.1. Has a doctor ever told you that you have diabetes?

☐ 0: No ***go to question 9.1***
1: Yes

8.2. How old were you when the doctor first told you that you had diabetes? *(Fill in one of the options below)*

Age ☐☐ (or) Year ☐☐☐☐ ☐ 99: Don't know

9. MYOPIA

9.1. Have you ever been told by a doctor or an optometrist that you need to wear glasses or contact lenses?

☐ 0: No ***go to question 10.1***
1: Yes

9.2. Did you get the glasses / contact lenses?

☐ 0: No ***go to question 10.1***
1: Yes

9.3. When did you first begin wearing glasses or contact lenses?

Age ☐☐ (or) Year ☐☐☐☐ ☐ 99: Don't know

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

9.4. What is the purpose for the glasses / contact lenses?

☐ 1: Seeing far ± Astigmatism
2: Seeing near ± Astigmatism
3: Seeing both far and near
4: Astigmatism only
99: Don't know

10. FAMILY HISTORY

10.1. Do you have a history of one of the following diseases in your first degree biological relatives (immediate family members)?

☐ 0: No, ***go to question 11***
1: Yes, ***specify in the following table.***

Code	First degree relatives
1:	Father
2:	Mother
10-19:	Sisters
20-29:	Brothers
30-39:	Sons
40-49:	Daughters

Code	Site of cancer
1:	Breast
2:	Ovarian
3:	Colorectal
4:	Others, specify _____ _____
99:	Don't know

Please use multiple rows if multiple diseases per individual
Pre-eclampsia = high blood pressure in pregnancy
Code Yes=1, No=0, Don't know=99 and N.A. for Not Applicable

First degree relative	Cancer		High blood pressure	Diabetes mellitus	Myopia	Cardio-vascular disease	Pre-eclampsia
	Yes=1 No=0 Don't know =99	Site	Yes=1 No=0 Don't know=99	Yes=1 No=0 Don't know=99	Yes=1 No=0 Don't know=99	Yes=1 No=0 Don't know=99	Yes=1 No=0 Don't know=99

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

11. MENSTRUAL CYCLES AND PREGNANCIES

11.1. Is your usual cycle regular, or has it varied by more than 5 days between periods in the last 6 months?

☐ 1: Regular ***go to question 11.2***
2: Varied by more than 5 days ***go to question 11.3***
3: Don't know ***go to question 11.3***

11.2. How long is your usual menstrual cycle between the start of one period and the start of the next period?

☐☐ days ☐ 99: Don't know

11.3. How old were you when you had your first period?

☐☐ years ☐ 99: Don't know

11.4. **IF THIS IS YOUR FIRST PREGNANCY, PLEASE GO TO QUESTION 12.1**

Next, would you please tell me the ending date(s) and outcome(s) of each of your pregnancy in sequence?

1: Live birth – Normal vaginal delivery
2: Live birth – Assisted delivery (Forceps/vacuum)
3: Live birth – Caesarean section
4: Abortion
5: Miscarriage
6: Stillbirth
7: Premature birth – Normal vaginal delivery
8: Premature birth – Assisted delivery
9: Premature birth – Caesarean section
10: Ectopic pregnancies
11: Others, please specify: _____

S/N	Pregnancy outcome	Year of start of pregnancy	Total weeks of pregnancy	Baby's weight (Specify in gm or in lb.oz)	If live birth, breastfed or not?		If breastfed, how long?			Pregnancy related complications						
										Hypertension		Diabetes Mellitus		Anaemia		Others (Please specify)
					0: No	1: Yes	Year(s)	Mth(s)	Wk(s)	0: No	1: Yes	0: No	1: Yes	0:No	1: Yes	
1																
2																
3																
4																
5																

11.5. Were you anaemic after the birth of any of your previous babies?

☐ 0: No
1: Yes
99: Don't know

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

12. MEDICATION

The questions below ask about REGULAR consumption of medications, supplements and traditional medicine in the past year BEFORE THIS PREGNANCY.

Regular refers to more than once a week for at least 1 month in past1 year.

12.1. Have you been taking any medications regularly before this pregnancy?

☐ 0: No ***go to question 12.2***
1: Yes, please specify in table below

S/N	Name of Medication
1	
2	
3	
4	
5	

12.2. Have you been taking folic acid supplement before your current pregnancy?

☐ 0: No ***go to question 12.3***
1: Yes

12.2.1. How many weeks before pregnancy have you been taking folic acid supplement?

☐☐ weeks

12.3. Are you still taking folic acid supplement **NOW**?

☐ 0: No
1: Yes

12.4. Have you been taking any fortified milk supplement (e.g. Anlene, Anmum) regularly before this pregnancy?

☐ 0: No
1: Yes

12.5. Have you been taking any probiotics (e.g. Yakult, Vitagen, Yoghurt) regularly before this pregnancy?

☐ 0: No
1: Yes

12.6. Have you been taking any other vitamins or supplements regularly before this pregnancy?

☐ 0: No
1: Yes

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

12.7. Have you been taking any traditional medicines regularly before this pregnancy?

☐ 0: No
1: Yes

13. INCOME

13.1. What is your personal monthly income?

☐ 1: $0 - $999
2: $1000 - $1999
3: $2000 - $3999
4: $4000 - $5999
5: more than $6000
6: Refuse to answer
99: Don't know

13.2. What is the monthly income of your household?

☐ 1: $0 - $999
2: $1000 - $1999
3: $2000 - $3999
4: $4000 - $5999
5: more than $6000
6: Refuse to answer
99: Don't know

Interview end time: _____

THANK YOU VERY MUCH FOR YOUR HELP.

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

GUSTO
GROWING UP IN SINGAPORE TOWARDS HEALTHY OUTCOMES

Week 26-28 clinic visit
Interviewer-administered questionnaire (Mother)

Study ID: _____ Date of interview: _____

Interviewer code: _____ Interview start time: _____

Have you changed your address or telephone number since you were seen in early pregnancy?

☐ 0: No
1: Yes: Please specify_____

Address:
Block/House no./Building Name/Street:

_____

Unit no:_____ Postal Code: _____

CONFIDENTIAL
PLEASE USE CAPITAL LETTER AND WRITE CLEARLY.

1. OCCUPATIONAL ACTIVITY

1.1. Have you had any jobs at any time since you became pregnant?

☐ 0: No *go to Section 2*

1: Yes

1.2. Would you please tell me your jobs during pregnancy and the weeks of your pregnancy in which you have done them?
If started before pregnancy, week started = 0
If job is still ongoing, week finished = 88

Occupation	Week started	Week finished
1.		
2.		
3		
4.		

1.3. How many hours in total did you work during an average week?

☐☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

1.4.Did this include working night shifts?
Night shift means "working at least once a week or more from 12 midnight to 6:00am"

☐ 0: No

1: Yes

1.5. At around this time, did your paid work involve any of the following activities in an average day at work?
i) Standing or walking for more than **four** hours in total?

☐ 0: No

1: Yes

ii) Kneeling or squatting for more than an hour in total?

☐ 0: No

1: Yes

iii) Standing or sitting with your trunk bent forward for more than an hour in total?

☐ 0: No

1: Yes

iv) Lifting or carrying weight of 25kg (56lbs) or more by hand (equivalent to a sack of potatoes, a nine year old child, a very heavy suitcase)

☐ 0: No

1: Yes

CONFIDENTIAL
PLEASE USE CAPITAL LETTER AND WRITE CLEARLY.

1.6. Have you at any time during your pregnancy left a job or changed the type of work that you were doing because of a health problem?

☐ 0: No
1: Yes

1.6a. If yes, give details of health problems_____
_____

1.6b. and the stage of pregnancy ☐☐ weeks

2. ACTIVITY AND EXERCISE– BEFORE THIS PREGNANCY

Now I'm going to ask you about your activity and exercise patterns during the 1 year before your pregnancy. We would like you to divide up a "typical" day into three types of activities. These are:
(1) sleeping or lying, (2) sitting, (3) standing or walking.

2.1. Over a typical 24 hour day, how many hours do you generally spend sleeping or lying with your feet up?
(ask what time she usually goes to bed & wakes up, including any at work!)

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

2.2. How many hours on a typical day do you spend sitting down?
(e.g. includes sitting at work, mealtimes, driving, reading, watching TV)

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

2.3. This would mean that you spend about xx hours a day on your feet.
Does this sound about right?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

Sum of hours reported in Q2.1, 2.2 and 2.3 should total up to 24 hours

Total hours: _____
Checked and signed: _____

2.4. Out of these xx hours spent on your feet, about how much of the time are you actively on the move (rather than standing fairly still)?

☐ 1: Very little 10%
2: Some 30%
3: About half 50%
4: Most 70%
5: Almost all 90%

2.5. During the 1 year before your pregnancy, how often have you done the following kind of exercises or activities?

a) **Strenuous exercise** which normally makes your heart beats rapidly AND leaves you breathless e.g. jogging, vigorous swimming or cycling, aerobics

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

b) **Moderate exercise** which normally leaves you exhausted but not breathless, e.g. brisk walking, dancing, easy swimming or cycling, badminton, sailing.

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

c) **Gentle exercise** which normally leaves you tired but not exhausted, e.g. walking, driving, housework (including washing windows and polishing), gardening, DIY, golf.

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

2.6.On a typical day, how many hours do you generally spend watching television?

☐ 1: More than 5 hours
2: 4-5 hours
3: 3-4 hours
4: 2-3 hours
5: 1-2 hours
6: Less than one hour
7: None

2.7. Which of the following best describes your walking speed?

☐ 1: Very slow
2: Stroll at an easy pace
3: Normal speed
4: Fairly brisk
5: Fast

3. ACTIVITY AND EXERCISE –DURING THIS PREGNACY

Can I now ask you about your activity and exercise patterns over the last 6 months?
As before, we would like you to divide up a "typical" day into three types of activities. These are:
(1) sleeping or lying, (2) sitting, (3) standing or walking.

3.1. Over a typical 24 hour day, how many hours do you generally spend sleeping or lying with your feet up?
(ask what time she usually goes to bed & wakes up, including any at work!)

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

3.2. How many on a typical day do you spend sitting down?
(e.g. includes sitting at work, mealtimes, driving, reading, watching TV)

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

3.3. This would mean that you spend about xx hours a day on your feet.
Does this sound about right?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

Sum of hours reported in Q3.1, 3.2 and 3.3 should total up to 24 hours

Total hours: _____
Checked and signed: _____

3.4. Out of these xx hours spent on your feet, about how much of the time are you actively on the move (rather than standing fairly still)?

☐ 1: Very little 10%
2: Some 30%
3: About half 50%
4: Most 70%
5: Almost all 90%

CONFIDENTIAL
PLEASE USE CAPITAL LETTER AND WRITE CLEARLY.

3.5. During the past six months, how often have you done the following kinds of exercise or activities?

a) **Strenuous exercise** which normally makes your heart beat rapidly AND leaves you breathless e.g. jogging, vigorous swimming or cycling, aerobics

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

b) **Moderate exercise** which normally leaves you exhausted but not breathless, e.g. brisk walking, dancing, easy swimming or cycling, badminton, sailing.

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

c) **Gentle exercise** which normally leaves you tired but not exhausted, e.g. walking, driving, housework (including washing windows and polishing), gardening, DIY, golf.

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

3.6.On a typical day, how many hours do you generally spend watching television?

☐ 1: More than 5 hours
2: 4-5 hours
3: 3-4 hours
4: 2-3 hours
5: 1-2 hours
6: Less than one hour
7: None

3.7. Which of the following best describes your walking speed?

☐ 1: Very slow
2: Stroll at an easy pace
3: Normal speed
4: Fairly brisk
5: Fast

4. CONTRACEPTION

4.1.How many weeks pregnant were you when you first found out that you were pregnant?

☐☐ wks

4.2.Was this pregnancy planned?

☐ 0: No *Go to question 4.4*
1: Yes: *Go to question 4.3*

4.3 If YES, did you change your diet when you were planning to be pregnant?

☐ 0: No *Go to question 5.1*
1: Yes *Go to question 5.1*

4.4.If NO, this pregnancy is due to

☐ 1: No contraception: *Go to question 5.1*
2: Failure of contraceptive methods

4.5.If NO, which was the main contraceptive method used which failed?

☐ 1. Safe period
2. Barrier e.g. condom, diaphragm
Hormones
3.a. Pills
3.b. Patch
3.c. Injection
3.d. Implants
4. Intrauterine contraceptive device
5. Withdrawal
6. Others: specify _____

5. DIET DURING PREGNANCY

5.1 Are you following any special diet?

☐ 0: No *go to question 5.3*
1: Yes

5.2 If yes, what is your special diet?

☐ 1: Vegetarian (Eggs and milk allowed)
2: Vegan (No eggs or milk allowed)
3. Diabetic diet
4. Low fat diet
5. Others, specify _____

5.3 How often do you eat eggs?

☐ 1: More than one egg a day
2: One egg a day
3. 4 to 6 eggs a week
4. 1 to 3 eggs a week
5. Less than one egg a week
6. Do not eat eggs at all

5.4 How often do you eat liver (any type e.g. chicken, beef, pork)?

☐ 1: Every day
2: 4 to 6 times a week
3. 1 to 3 times a week
4. Less than once a week but more than once a month
5. Less than once a month
6. Do not eat liver at all

5.5 How often do you eat out or purchase take-away foods?

☐ 1: Two meals a day or more
2: One meal a day
3. 4 to 6 meals a week
4. 1 to 3 meals a week
5. Less than once a week
6. Never/ rarely

5.6 I would like to find out more about your diet during pregnancy compared to what you usually ate before you were pregnant. I will be asking you about your eating habit for a list of foods during pregnancy. Please tell me if you ate more, less or similar amount of the food during pregnancy compared to your usual diet.

	Types of Food	**Change in amount**
1.	Chicken	
2.	Fish	
3.	Meat (beef / mutton / pork)	
4.	Organ meats (e.g. liver, kidney, heart, brain)	
5.	Seafood (e.g. prawn, crab, mussels, clams)	
6.	Egg	
7.	Vegetables (all types)	
8.	Fruits (all types)	
9.	Red, orange, yellow fruits and vegetables (e.g. carrots, papaya)	
10.	Rice, noodles, breads	
11.	Cheese, yogurt	
12.	Chocolates, sweets, biscuits, cakes	
13.	Milk	
14.	Chocolate drinks (Milo, Ovaltine)	
15.	Soft drinks (e.g. Coke, sprite, 7-up, Pepsi)	
16.	Tea	
17.	Coffee	
18.	Wine/alcohol (including tonic wine)	

Key
1: More
2: Less
3. Same as before
9. Don't usually eat

6. APPETITE AND NAUSEA DURING PREGNANCY

6.1. Have you experienced any nausea or sickness since becoming pregnant?

☐ 0: No *go to question 6.5*
1: Yes

6.2. If yes, has this been:

☐ 1: Mild (nausea only)
2: Moderate (sometimes sick, vomiting)
3: Severe (regularly sick, vomiting, can't retain meals)

6.3. If yes, were you admitted to the hospital because of nausea?

☐ 0: No *go to question 6.5*
1: Yes

6.4. If yes, how were you treated?

☐ 1: Fasting, then slowly introducing food
2: Intravenous fluid treatment
3: Medication *(Note: Refer to medical records/CPSS)*

6.5. **Compared with BEFORE you were pregnant**, are you eating:

☐ 1: More *go to question 6.5a*
2: The same *go to question 7.1*
3: Less in amount *go to question 6.5b*
99: Don't know

6.5a. If **more**, is this:

☐ 1: Because you feel more hungry
2: To prevent from feeling sick
3: Because you feel it is best for the baby
4: Other reasons; specify: _____

6.5b. If **less**, is this:

☐ 1: Because you feel less hungry
2: Because of nausea/sickness
3: Don't want to put on too much weight
4: Other reasons; specify: _____

7. DIETING

7.1 Which of the following describes you best?

☐ 1: I have NEVER been on a diet to lose weight.
2: I have ONLY ONCE been on a diet to lose weight.
3: I USED TO diet REGULARLY to lose weight but NOT ANYMORE
4: I go on a diet to lose weight EVERY NOW AND AGAIN.
5: I am USUALLY on a diet to lose weight.

If answered 2, 4, 5, please ask question 7.2; otherwise go to next section.

7.2 Are you currently trying to lose weight by dieting?

☐ 0: No
1: Yes

8. ALCOHOL CONSUMPTION – BEFORE THIS PREGNANCY

I'd like to ask you a few questions about your drinking and smoking habits.

8.1 Did you ever drink alcohol before this pregnancy?

☐ 0: No *go to section 9*
1: Yes
99: Don't know

8.2 How often did you drink the following alcoholic beverages in the 1 year before you became pregnant? Please select the category that best describes how often and how much you drank during the past year.

Alcoholic beverages	Average consumption in past year	Usual serving size
Beer	☐ 1. Never or hardly ever 2. Once a month 3. 2-3 times a month 4. Once a week 5. 2-3 times a week 6. 4-6 times a week 7. Once a day 8. 2 or more times a day	☐ 1. One small bottle (375ml) or less 2. One large bottle (750ml) 3. Two large bottles 4. Three large bottles or more
Wine (eg. red wine)	☐	☐ 1. One wine glass (118ml) or less 2. Two wine glasses 3. Three wine glasses 4. Four wine glasses or more
Traditional wine (eg. DOM)	☐	☐ 1. One wine cup (30ml) or less 2. Two wine cups 3. Three wine cups 4. Four wine cups or more
Hard liquor (eg. brandy)	☐	☐ 1. One drink (30ml) or less 2. Two drinks 3. Three drinks 4. Four drinks or more

9 ALCOHOL CONSUMPTION – DURING THIS PREGNANCY

Did you ever drink alcohol during this pregnancy?

☐ 0: No *go to section 10*
1: Yes
2: Refuse to answer

9.1 During the past 6 months, how often did you drink the following alcoholic beverages? Please select the category that best describes how often and how much you drank.

Alcoholic beverages	Average consumption past 6 mth	Usual serving size
Beer	☐ 1. Never or hardly ever 2. Once a month 3. 2-3 times a month 4. Once a week 5. 2-3 times a week 6. 4-6 times a week 7. Once a day 8. 2 or more times a day	☐ 1. One small bottle (375ml) or less 2. One large bottle (750ml) 3. Two large bottles 4. Three large bottles or more
Wine (eg. red wine)	☐	☐ 1. One wine glass (118ml) or less 2. Two wine glasses 3. Three wine glasses 4. Four wine glasses or more
Traditional wine (eg. DOM)	☐	☐ 1. One wine cup (30ml) or less 2. Two wine cups 3. Three wine cups 4. Four wine cups or more
Hard liquor (eg. brandy)	☐	☐ 1. One drink (30ml) or less 2. Two drinks 3. Three drinks 4. Four drinks or more

10. PERSONAL VIEWS ON BREAST FEEDING

10.1 Have you breastfed before?

☐ 0: No, *go to question 10.3*
1: Yes

10.1.1 If "YES", how many children have you breastfed before?

☐☐ Number of children

10.1.2 If YES, please describe your type of breastfeeding for your last child:

☐ 1. Exclusive breastfed (Only breast milk with no water)
2. Predominant breastfed (Breast milk and liquids (including water) other than formula)
3. Partial breastfed (Breast milk, formula and liquids)

10.1.3 How long did you breastfeed your last child?

☐ Year ☐☐ Months ☐☐ weeks

10.2 Are you still breastfeeding during this pregnancy?

☐ 0: No
1: Yes but I stopped at ☐☐ weeks of pregnancy
2: Yes, I am still continuing breastfeeding

10.3 Do you know people who have successfully breastfed their babies?

☐ 0: No
1: Yes

10.4 Did you receive advice from family or friends about breastfeeding?

☐ 0: No
1: Yes

10.5 Have you read books or watched programs on breastfeeding?

☐ 0: No
1: Yes

10.6 Are you currently attending antenatal classes?

☐ 0: No
1: Yes

10.7 Do you plan to breastfeed?

☐ 0: No
1: Yes, for how long ☐☐ months , *go to question 10.8*
99: Don't know

10.7.1 If No, please specify reason

☐ 1: Underlying medical problems
2: Painful
3: Troublesome
4: Inconvenient
5: Formula more nutritious
6: No reason
7: Others, specify _____

10.8 Who will be the main person helping you with the baby after delivery?

☐ 1. Confinement nanny
2. Mother / Mother-in-law
3. Husband
4. Other relatives
5. Others, specify_____

11. SMOKING – <u>BEFORE</u> THIS PREGNANCY

11.1 Have you ever smoked regularly (at least once a day for a year or more)?

☐ 0: No *go to question 11.5*
1: Yes
2: Refuse to answer

11.2 How old were you when you first smoked regularly?

☐☐ yrs

11.3 Did you smoke during the 1 year before you became pregnant?

☐ 0: No *go to question 11.5*
1: Yes

11.4 If yes, how many sticks per day? *Record maximum stated.*

☐☐

<u>Note to interviewer:</u> You may want to explain to the participant that even though she does not smoke, there is some evidence of health implications from second-hand smoke exposure. The following questions are to capture information on second-hand smoke exposure, i.e. where the participant was close enough to the smoker(s) to smell the smoke.

11.5 Did anyone living in your home smoke at home on a daily basis for 6 months or longer?

☐ 0: No *go to question 11.7*
1: Yes

11.6 For how many years did at least 1 person living in your home smoke daily at home?

☐ 1: 1 year or less
2: 2-5 years
3: 5-14 years
4: 15-24 years
5. 25+ years

11.7 Have you ever had a job in which, on a daily basis, you were exposed to cigarette smoke from others?

☐ 0: No
1: Yes

12. SMOKING – DURING THIS PREGNANCY

12.1 Are you currently smoking?

☐ 0: No *go to question 12.3*
1: Yes

12.2 If yes, how many sticks per day? *Record maximum stated.*

☐☐

12.3 During your pregnancy, did anyone living in your home smoke at home on a daily basis?

☐ 0: No
1: Yes

12.4 During your pregnancy, have you ever had a job in which, on a daily basis, you were exposed to cigarette smoke from others?

☐ 0: No *go to section 13*
1: Yes

12.5 On average, how many hours were you exposed to cigarette smoke at work?

☐ 1: 1 hour or less
2: 1-3 hours
3: More than 3 hours

12.6 Are you currently exposed to cigarette smoke at work on a daily basis?

☐ 0: No
1: Yes

13. MEDICATION

13.1 Are you taking any medications / supplements / traditional medicine regularly DURING this pregnancy?

Regular refers to more than once a week

☐ 0: No *END OF QUESTIONNAIRE*
1: Yes, please specify in table below

S/N	Name of Medication
1	
2	
3	
4	
5	
S/N	**Name of Supplement**
1	
2	
3	
4	
5	
S/N	**Name of Traditional Medicine**
1	
2	
3	
4	
5	

THANK YOU VERY MUCH FOR YOUR HELP!

Interview end time: _____

Appendix 3

Edinburgh Postnatal Depression Scale[1] (EPDS)

Study ID: _____ Date: _____

As you are pregnant or have recently had a baby, we would like to know how you are feeling. Please check the answer that comes closest to how you felt **IN THE PAST 7 DAYS**, not just how you feel today.

Here is an **example**, already completed.

I have felt happy:

	1. Yes, all the time
×	2. Yes most of the time
	3. No, not very often
	4. No, not at all

This would mean: "I have felt happy most of the time" during the past week. Please complete the other questions in the same way.

In the past 7 days:

1. I have been able to laugh and see the funny side of things

	1. As much as I always could
	2. Not quite so much now
	3. Definitely not so much now
	4. Not at all

2. I have looked forward with enjoyment to things

	1. As much as I ever did
	2. Rather less than I used to
	3. Definitely less than I used to
	4. Hardly at all

3. I have blamed myself unnecessarily when things went wrong

	1. Yes, most of the time
	2. Yes, some of the time
	3. No, not very often
	4. No, never

[1] source: Cox JL, Holden JM and Sagovsky R. 1987. Detection of postnatal depression: Development of the 10-item Edinburgh Postnatal Depression Scale. British Journal of Psychiatry 150: 782 -786.

4. I have been anxious or worried for no good reason

- [ ] 1. No, not at all
- [ ] 2. Hardly ever
- [ ] 3. Yes, sometimes
- [ ] 4. Yes, very often

5. I have felt scared or panicky for no very good reason

- [ ] 1. Yes, quite a lot
- [ ] 2. Yes, sometimes
- [ ] 3. No, not much
- [ ] 4. No, not at all

6. Things have been getting on top of me

- [ ] 1. Yes, most of the time I haven't been able to cope at all
- [ ] 2. Yes, sometimes I haven't been coping as well as usual
- [ ] 3. No, most of the time I have coped quite well
- [ ] 4. No, I have been coping as well as ever

7. I have been so unhappy that I have had difficulty sleeping

- [ ] 1. Yes, most of the time
- [ ] 2. Yes, sometimes
- [ ] 3. Not very much
- [ ] 4. No, not at all

8. I have felt sad or miserable

- [ ] 1. Yes, most of the time
- [ ] 2. Yes, quite often
- [ ] 3. Not very often
- [ ] 4. No, not at all

9. I have been so unhappy that I have been crying

- [ ] 1. Yes, most of the time
- [ ] 2. Yes, quite often
- [ ] 3. Only occasionally
- [ ] 4. No, never

[1] source: Cox JL, Holden JM and Sagovsky R. 1987. Detection of postnatal depression: Development of the 10-item Edinburgh Postnatal Depression Scale. British Journal of Psychiatry 150: 782 -786.

10. The thought of harming myself has occurred to me

☐	1. Yes, quite often
☐	2. Sometimes
☐	3. Hardly ever
☐	4. Never

Total Score: _____

[1] source: Cox JL, Holden JM and Sagovsky R. 1987. Detection of postnatal depression: Development of the 10-item Edinburgh Postnatal Depression Scale. British Journal of Psychiatry 150: 782 -786.

Appendix 4

Anthropometric measurements for participants (Parents and children)

General note:

Length, height, circumferences, and skinfolds are to be recorded to the nearest unit specified for each measure.

Arm circumference and skinfold measurements are taken on the right side of the body, except for extenuating circumstances.

Recumbent Length (For 18 and 24 month)

- The measurements are taken in **duplicate**.
- A third measurement should be taken if the first two measurements differed by **>1.0 cm**.
  Note: *If it is necessary to take a third measurement, the two closest measurements are averaged. Should the third measurement fall equally between the first two measurements, all three should be averaged.*
- A SECA infant mat is used for measurement of recumbent length. The mat has a fixed headpiece, horizontal backboard, and movable foot piece.
- Ask the parent or guardian to remove the child's clothes except for a diaper or underpants and t-shirt.
- Position the child on the infant mat with the feet positioned against the foot piece and the head against the headpiece.
- Note: Children often cry when placed on the infantometer, so ask the parent or guardian to stand beside the examiner, make eye contact, and reassure the child.
- One person supports the child's head and ensures that the head is positioned in the Frankfort horizontal plane. To do this, apply gentle traction to bring the top of the head in contact with the fixed headpiece. Secure the child's head in the proper alignment by lightly cupping the palms of your hands over the ears.
- A second person can align the child's legs by placing one hand gently but with mild pressure over the knees. With the other hand, slide the foot piece to rest firmly against the soles of the feet. The toes should point upward with both soles of the feet flexed perpendicular against the acrylic foot piece. To encourage the child to flex the feet, run the tip of your finger down the inside of the foot.

Height

- Standing height is measured from the top of the participant's head to his or her heels.
- Remove shoes, hair ornaments and undo braids and buns.
  Note: *Adjustments for shoes and hair:*
  When participants cannot remove hair braids, buns, and headwear that interferes with the stature measurement, measure the distance from the scalp to the top of the hair with a small ruler to the nearest 0.1 cm.
  If shoes are worn, measure the height of the shoe heel to the nearest 0.1 cm. A corrected height value can be calculated by subtracting these distances from the original stature measurement, thus yielding an adjusted stature value
- Ask the participant to stand erect against the backboard with the body weight evenly distributed and both feet flat on the stadiometer platform.
- Keep the legs straight.
- The participant's feet should be positioned with the heels together and toes pointed slightly outward at approximately a 60 degree angle.
- Check to be sure that the back of the head, upper back between shoulder blades, buttocks, and heels touching the vertical stand/board of the stadiometer.
  Note:
  Depending on the overall body conformation of the individual, all four contact points - head, shoulders, buttocks, and heels - may or may not touch the stadiometer backboard. In such instances it is important to obtain the best measurement possible.
- The observer should get to a face-to-face level with the subject and positioned her head so that a horizontal line drawn from the ear canal to the lower edge of the eye socket, running parallel to the baseboard (i.e., the Frankfort plane positioned horizontally).
- The headboard is pulled down to rest firmly on top of the head and compressed the hair.
- A gentle push applied to the tummy can help the subject stand to full height.
- Instruct the survey participant to stand as tall as possible, take a deep breath, and hold this position.
  Note: *The act of taking a deep breath helps straighten the spine to yield a more consistent and reproducible stature measurement. Notice that the inhalation will cause the headpiece to rise slightly.*
- As soon as the participant inhales, record the measurement. The reading is taken to the nearest 1 mm.Measurements are taken in duplicate. A third measurement should be taken if the first two measurements differed by >1.0 cm.
  Note: *If it is necessary to take a third measurement, the two closest measurements are averaged. Should the third measurement fall equally between the first two measurements, all three should be averaged.*

Weight

- Measurements are taken in duplicate.
- A third measurement should be taken if the first two measurements differ by **>200 grams**.
  Note: If it is necessary to take a third measurement, the two closest measurements are averaged. Should the third measurement fall equally between the first two measurements, all three should be averaged.
  Participants are asked to remove objects such as cell phones, wallets, and toys from their pockets.
- In light clothings and without shoes, the participant to stand in the center of the scale platform with hands at their sides and looking straight ahead.
- Measure to **last 0.1 kg.**

Head circumference

The child may need to be held by the parent or a health professional while the examiner uses a tape measure to measure the child's head.

- The measurements are taken in duplicate.
- A third measurement should be taken if the first two measurements differed by **>1.0 cm**. If it is necessary to take a third measurement, the two closest measurements are averaged. Should the third measurement fall equally between the first two measurements, all three should be averaged.
- The circumference of the head is measured on children from birth through 36 months of age.
- Follow the steps below to obtain the head circumference measurement:
  1. Position of the study participant: Instruct the parent (or guardian) to stand holding the child over the parent's left shoulder or else sit with the child in the parent's lap. Ask the parent to remove hair ornaments or braids that might interfere with the measurement.
  2. Taking the measurement: Place the head circumference tape around the child's head so that the tape lies: across the frontal bones of the skull; slightly above the eyebrows; perpendicular to the long axis of the face; above the ears; and over the occipital prominence at the back of the head. Move the tape up and down over the back of the head to locate the maximal circumference. Tighten the insertion tape so that it fits snugly around the head and compresses the hair and underlying soft tissues. Measure the circumference to the nearest 0.1 cm.
  3. Record the result. Remove the head circumference tape

Mid upper arm circumference (MUAC)

- The mid-upper-arm point is half the distance between the acromion process (the most lateral bony protuberance of the back of the shoulder) and the olecranon (the bony structure that stands out when the elbow is bent).
- The midpoint was located for measurement of the mid-upper-arm circumference (MUAC) and triceps skinfold thickness.
- Direct the participant to turn away from you.

- Ask participant to stand upright with his/her weight evenly distributed on both feet, the right arm bent 90 degrees at the elbow, and the right palm facing up.
- The observer palpates the shoulder to find the acromion. Locate the end of the spine of the right scapula by following the scapula out to the arm until it makes a sharp V-turn to the front of the body.
- Using the cosmetic pencil/pen, make a horizontal line on the uppermost edge of the posterior border of the spine extending from the acromion process.
- The observer placed the zero point of the tape on the mark over the acromion process and runs it downward along the back of the arm to the tip of the olecranon process, the bony part of the elbow.
- The tape must be centered on the posterior surface of the arm.
- The midpoint on the posterior aspect of the arm is marked.
- Finally, tell the participant to relax the right arm.
- Proceed to the arm circumference measurement.
- For measurement of the MUAC, the arm hangs in a relaxed position or is held in the extended position; care should be taken not to flex or tighten the muscles which will yield an inaccurate measurement.
- The tape is then wrapped around the arm over the marked midpoint. Position the tape perpendicular to the long axis of the upper arm and make sure the tape is level around the circumference. The tape has to lie flat around the arm.
- Pull the two ends of the overlapping tape together so that the zero end sits below the measurement value and the result lies on the lateral aspect of the arm (not the posterior surface).
- Check that the tape fits snug around the arm but does not compress the skin. Take the measurement to the **nearest 0.1 cm.**

Abdominal/Waist circumference

(National Health and Nutrition Examination Survey [NHANES])

- Locate the top of the right iliac crest.
- With the cosmetic pencil/pen, draw a horizontal line just above the uppermost lateral border of the right iliac crest.
- Cross this mark at the midaxillary line, which extends from the armpit down the side of the torso.
- Repeat the same process on the participant's left side.
- Make sure the participant does not inhale while his/her waist circumference is being measured (at expiration) and that the tape is not twisted.
- Wrap the tape measure around the individual's waist as you would a belt, making sure that the zero end of the measure is at the beginning of the circumference.
- Be sure to position the tape in a horizontal plane at the level of the measurement mark.
- Check the horizontal alignment of the tape before taking the measurement and make sure the tape lies snug but does not compress the skin.
- Note: If another person is available, the person can be positioned on the opposite side of the participant and should check that the tape sits parallel to the floor and

lies snug but does not compress the skin. Always position the zero end of the tape below the section containing the measurement value.

- Take the measurement to the **nearest 0.1 cm** at the end of the participant's normal expiration. Measurements are taken in duplicate.
- A third measurement should be taken if the first two measurements differed by >1.0 cm for parents or > 0.5 cm for children up to 11 yrs
- If it is necessary to take a third measurement, the two closest measurements are averaged. Should the third measurement fall equally between the first two measurements, all three should be averaged.

Hip Circumference (Mothers)

- Participant should be wearing an examination gown or in thin underwear when this measure is taken.
- The hip girth measurement should be made on the participant's right side (rather than in front) with the patient's feet together.
- Hip girth is measured at the level of the symphysis pubis anteriorly and posteriorly at the level of the maximal protrusion of the gluteal muscles (Exhibit 1). This level usually is the greatest circumference of the hips, around the greater trochanter (but not always).
- Mark the level of greater trochanter. (Marking may not be possible if the mothers do not agree to lower the garment. However this should be the exception and not the rule)
- Keep the anthropometric tape horizontal at this level and record the measurement to the nearest 0.1 cm.
- Measurements are taken in duplicate.
- A third measurement should be taken if the first two measurements differed by >1.0 cm for parents or > 0.5 cm for children up to 11 years.
- If it is necessary to take a third measurement, the two closest measurements are averaged.
- Should the third measurement fall equally between the first two measurements, all three should be averaged.

Skinfold thickness

- Measurements are to be taken in **triplicates and record to last completed 0.2 mm.**
- If repeated tests vary by **more than 1mm, repeat the measurement**.
- The final value recorded should be the average of the three that seems to best represent the skinfold site.

Biceps-

- Mark the anterior surface of the biceps midway between the anterior auxiliary fold and the antecubital fossa (anterior auxiliary line is the crease where the top of the arm, when hanging down, meets the chest).
- Grasp a vertical fold 1 cm above the mark with arm relaxed and hanging by side, straight.
- The caliper jaws were applied at right angles to the "neck" of the fold just below the finger and thumb over the midpoint mark.
- While maintaining a grip on the skinfold, the observer gently released the caliper handles and allowed the jaws to close on the fat fold for two seconds before taking the reading to the last completed 0.2 mm.

Triceps-

- Along the midline on the back of the triceps of the right arm, determine the midpoint located between the top of the acromial process (top of the shoulder) to the bottom of the olecranon process of the ulna (elbow). (This midpoint has already been marked before measurement of mid upper arm circumference).
- The elbow should be extended and the arm relaxed.
- Pinch the skin so that the fold is running vertically.
- Picked up the skinfold about 1 cm above the midpoint mark over the triceps muscle, with the fold running downward along the midline of the back upper arm.
- The caliper jaws were applied at right angles to the "neck" of the fold just below the finger and thumb over the midpoint mark.
- While maintaining a grip on the skinfold, the observer gently released the caliper handles and allowed the jaws to close on the fat fold for two seconds before taking the reading to **the last completed 0.2 mm**

Subscapular-

- The measurement point for the subscapular skinfold located immediately below the inferior angle of the scapula was identified by palpating and marking the inferior angle of the scapula.
- The subject stands or sits with shoulders relaxed or gently held down to prevent movement of the scapula.
- The skinfold was picked up 1 cm above and medial to the subscapular mark, where the fold is taken on the diagonal line coming from the vertebral border to between 1 and 2 cm from the inferior angle of the scapulae, so that the fold ran diagonally down toward the left elbow.
- The caliper was applied to the "neck" of the fold over the mark

Suprailiac-

- A diagonal fold above the crest of the ilium at the spot where an imaginary line would come down from the anterior auxiliary line.
- Determine the anterior auxiliary line and palpate for the iliac crest (top of the hip bone).
- Grasp the skin that follows the natural fold which will follow a line of approximately from the suprailiac to just below the umbilicus (bellybutton), an angle of approximately 30 degrees.

MANUAL OF PROCEDURES
Procedures for using BPro and A-PULSE CASP

Aim: To derive the central aortic systolic pressure, radial augmentation index (rAI) and a range of other pulse wave indices from the radial pulse.

Applicability:
All ground staff involved in clinic activities of GUSTO study

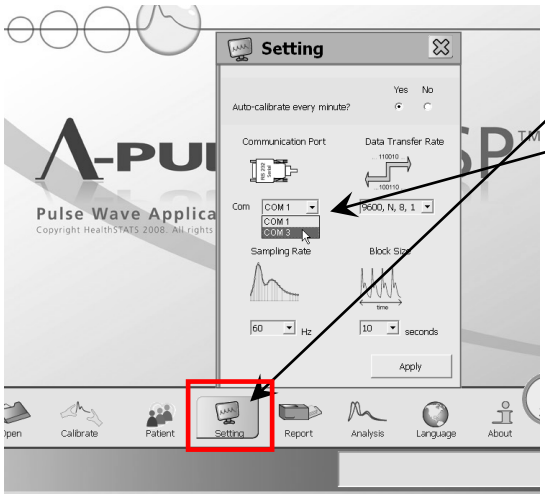
Location:
Antenatal Clinics of Department of Obstetrics and Gynecology, KKH and NUH

Step-by-step procedures:

1) Launching A-PULSE CASP

Open the start menu and select All Programs. Select A-PULSE CASP and click to launch the program. Alternatively, you can click the shortcut on the desktop for quick access.

2) Configuring the BPro USB data cable for A-PULSE CASP



1. Click Settings on the A-PULSE CASP startup screen to open the Settings dialog box.
2. Click the drop-down button for Com to see the initial port number present.
3. Plug the USB connector of the BPro data cable into the USB port of the computer. Click Settings on the A-PULSE CASP startup screen to open the Settings dialog box.
4. Click the drop-down button for Com to select the NEW port number that is different from the initial ones present.

Note:
• You must configure the COM port of the BPro USB data cable in order for A-PULSE CASP to communicate with the BPro.
• You may need to reconfigure the COM port of the BPro USB data cable once you exit A-PULSE CASP.

3) Activating BPro for use with A-PULSE CASP

1. Click the start button (first button) on the A-PULSE CASP startup screen to open the pulse wave window.

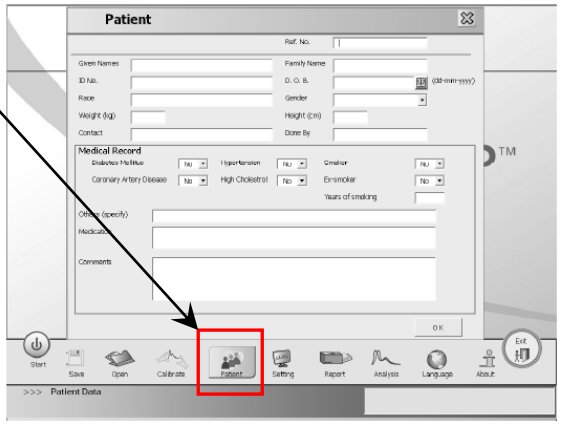
2. Press and hold the Mode/Set (MODE) and +/Std by (FORWARD) buttons (top right & bottom left button simultaneously) on the BPro until POS AdJ appears.
3. The activation is now complete. Try tapping the BPro to see waveforms on the window. The BPro is ready to capture pulse waveforms for display by A-PULSE CASP.

4) Taking calibration readings

1. Have the patient sit comfortably with the blood pressure cuff on the patient arm. The green marker of the cuff should be centered over the brachial artery on the inside of the arm, and the bottom edge should be about 2 to 3 cm above the elbow.
2. Rest the patient's arm on the table such that the cuff is at the same level as the heart.
3. Ask the patient to relax for at least 10 minutes before taking the first blood pressure measurements.
4. Take 3 blood pressure measurements on the same arm, with an interval of 30 to 60 seconds between measurements, and record the systolic and diastolic readings.
5. If any of the readings for systolic or diastolic pressure differ by more than 10 mmHg, take more measurements until the last 3 consecutive readings for each pressure do not differ from each other by more than 10 mmHg.
6. Average the 3 readings for each pressure and use the average systolic and diastolic readings for calibration.

5) Entering patient data (while measuring brachial pressure)

1. Click Patient on the toolbar of the A-PULSE CASP startup screen to open the Patient dialog box.



2. Enter all the necessary data e.g. ID No. (as GUSTO study ID), date of birth, gender and click OK.

6) Calibrating and capturing arterial pulse waveform

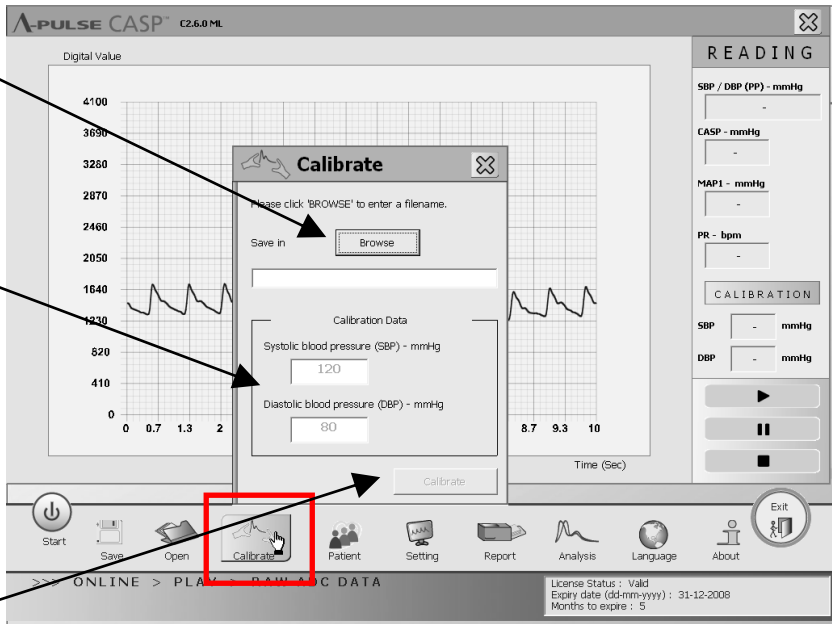
1. Using the plunger positioning marker (white vertical line) as a guide, place the plunger of the BPro over the radial artery where the strongest pulse is felt.
   ***Note**: This should be the same arm used for measuring brachial pressure earlier*

2. Hold the plunger in place and fasten the BPro firmly to the wrist.
3. Once there's a constant waveform, click Calibrate on the toolbar to open the Calibrate dialog box.

4. Click Browse in the Calibrate dialog box. A Save As dialog box will appear. Save the file.

5. In the Calibrate dialog box, enter the average systolic reading in the Systolic blood pressure (SBP) and the average diastolic reading in the Diastolic blood pressure (DBP).

6. Ask the patient to keep his or her arm as still as possible, and then click Calibrate.



7. The calibration is now in progress.

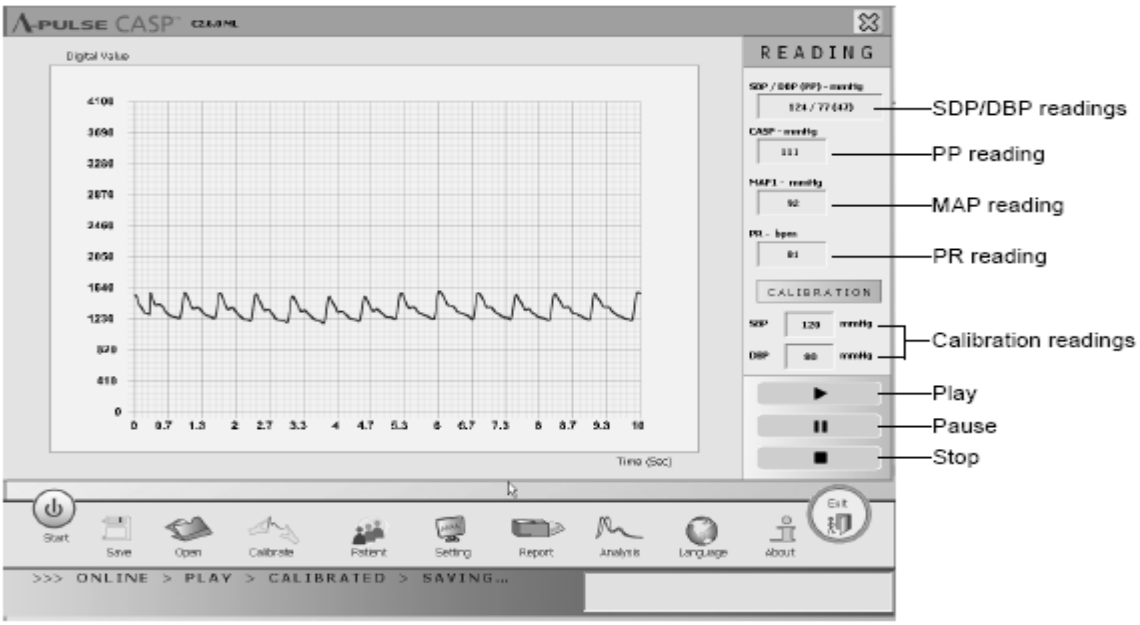
Note:

• Ask the patient to continue keeping his or her arm as still as possible throughout the whole process of calibrating and capturing the arterial pulse waveform.

• After clicking Calibrate, the arterial pulse waveform will appear in the plot area. A-PULSE CASP will begin the calibration process and complete it in about 10 seconds, and save the waveform data on the computer.

8. The calibration readings will appear in the CALIBRATION pane. The ONLINE > PLAY > CALIBRATED > SAVING … message will appear on the status bar to indicate that the waveform is being saved to the specified location.

9. Calibrated readings will appear in the READINGS pane about 20 seconds later.



10. Continue reading the waveform for another 40 seconds (4 rounds).

11. To stop saving the data, click the stop icon.

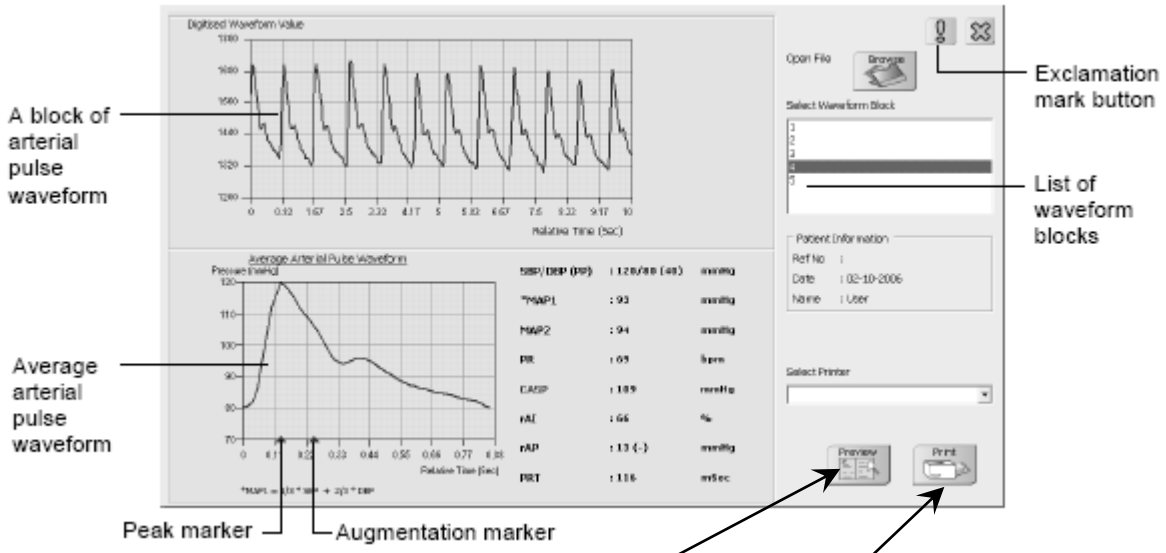
7) <u>Managing A-PULSE CASP reports</u>

1. Click the Report icon on the toolbar of the A-PULSE CASP startup screen to open the average arterial pulse waveform window.

2. Click Browse to open the Open dialog box.

3. Click to select the correct subject's file and click Open to open the file. A list of waveform blocks will appear in the Select Waveform Block window.

4. Click the block of waveform you want to display. The selected block of waveform and an average arterial pulse waveform will be displayed in the respective plot areas.



5. Click the Preview button to view the report. To print the report after previewing, click Print in the PRINT PREVIEW window

6. To print the report without previewing, click Print in the Average Arterial Pulse Waveform window.

8) Waveform analysis report sample

HealthSTATS
HealthSTATS International Pte. Ltd.

Ref. No:

Arterial Pulse Waveform Analysis

Date: 02-10-2006

Given Names: User — Family Name:

ID No.: — DOB:

Race: — Gender: Male

Weight (kg): — Height (cm):

Contact: — Done By:

Medical Record

☐ Diabetes Mellitus ☐ High Cholestrol

☐ Coronary Artery Disease ☐ Smoker

☐ Hypertension ☐ Ex-Smoker _____ years

Others (specify):

Medication:

Comments:

Reviewed by: Signature: Date:

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HealthSTATS International Pte. Ltd.

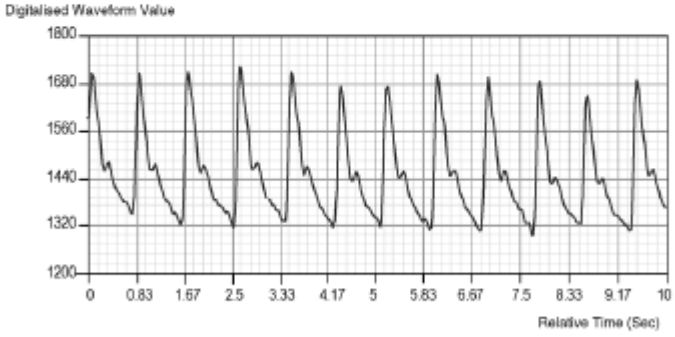
Ref. No:

Arterial Pulse Waveform Analysis

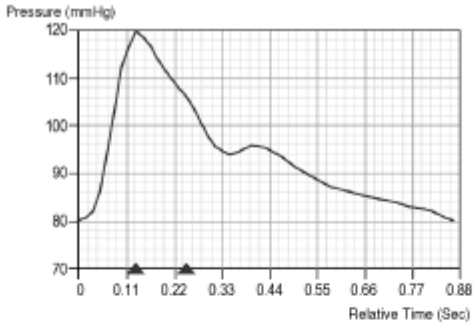
Date: 02-10-2006

Given Names: User Family Name:

Arterial Pulse Waveform



Average Arterial Pulse Waveform



SBP:	120	mmHg
DBP:	80	mmHg
PP:	40	mmHg
MAP1 :	93	mmHg
MAP2 :	94	mmHg
PR:	69	bpm
CASP:	109	mmHg
rAI:	66	%
rAP:	14 (-)	mmHg
PRT:	117	mSec

Explanation of Terms

SBP	Systolic blood pressure of the average single waveform.
DBP	Diastolic blood pressure of the average single waveform.
PP	Pulse pressure. The difference between SBP and DBP.
MAP1	Mean arterial pressure (MAP1 = 1/3 * SBP + 2/3 * DBP).
MAP2	Time-weighted average area under the curve.
PR	Pulse rate.
CASP	Derived central aortic systolic pressure.
rAI	Radial augmentation index.
rAP	Radial augmentation pressure.
PRT	Relative time between SBP and augmentation.

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Appendix 6

MOP for measuring Blood Pressure by Dynamap CARESCAPE™ V100

General Instruction for both Mother and Child

- The measurement has to be done on the right side of the upper arm.
- Have the mother/child sits quietly for a period of five minutes before the first BP is taken.
- Ensure the subject is seated, legs uncrossed, in a quiet room, with the elbow and forearm resting comfortably on the armrest of the chair or table, with the palm of the hand turned upward.
- The area to which the cuff is to be applied must be bare (free of clothing).
- Accuracy of measurement depends on the use of appropriate cuff size (GE CRITIKON BP cuffs) *(Fig 1 and Table 1).*

Figure 1

Table 1

Size	Colour	Limb Circumference	
Infant	Orange/White	8-13 cm	Child
Child	Green/White	12-19 cm	
Small Adult	Light Blue/White	17-25 cm	
Small Adult Long	Light Blue/White	17-25 cm	
Adult	Navy/White	23-33 cm	Mother
Adult Long	Navy/White	23-33 cm	
Large Adult	Rose/White	31-40 cm	
Large Adult Long	Rose/White	31-40 cm	
Thigh	Brown/White	38-50 cm	

- Wrap the cuff snuggly around the subject's upper arm allowing a finger between the child's arm and the cuff. Do not apply cuff to areas when skin is not intact or tissue is injured. *(Figure 2)*
- The palm of the subject's hand should turn upwards, making sure the long edges of the cuff lie on top of each other as the cuff is wrapped around.

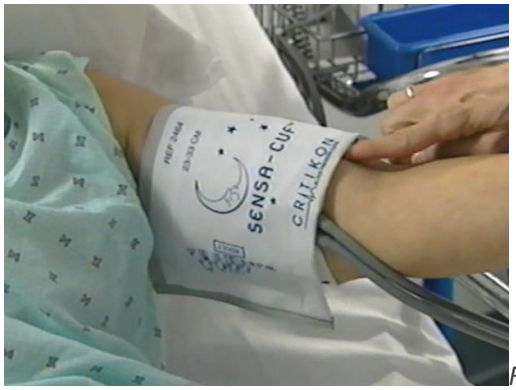

Figure 2

- The ***dotted index line*** and the ***solid range line*** on the cuff for easier and accurate placement. *(Figure 3A and 3B)*

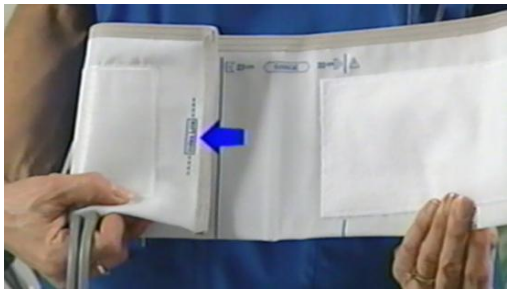

Figure 3A

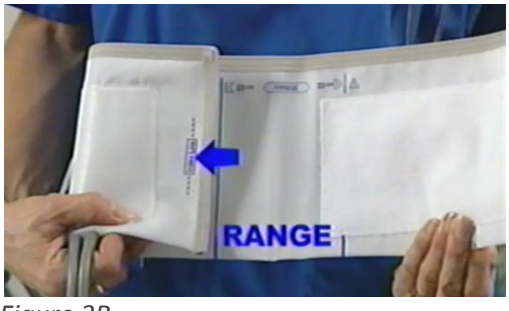

Figure 3B

- The dotted index line needs to fall within the solid range line. If the index line goes beyond the solid line, use a larger cuff. If the index line stops before the solid range line, use a smaller cuff.

Taking the blood pressure measurement

- To get started turn on the monitor by pressing **ON/OFF** button *(Figure 4).*

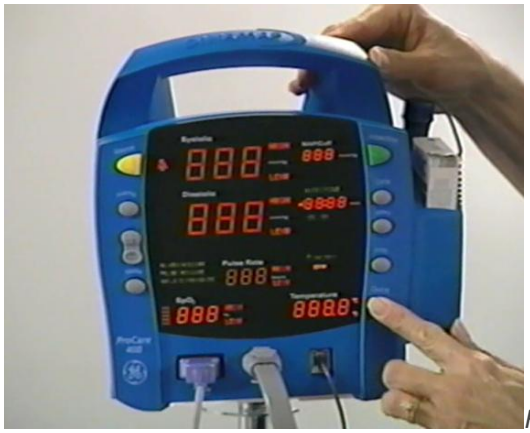

Figure 4

- Apply the blood pressure cuff to the subject's upper arm.
- Connect the cuff to the air hose.
- Press **INFLATE/STOP** button on the monitor and wait for the output *(Figure 5)*.

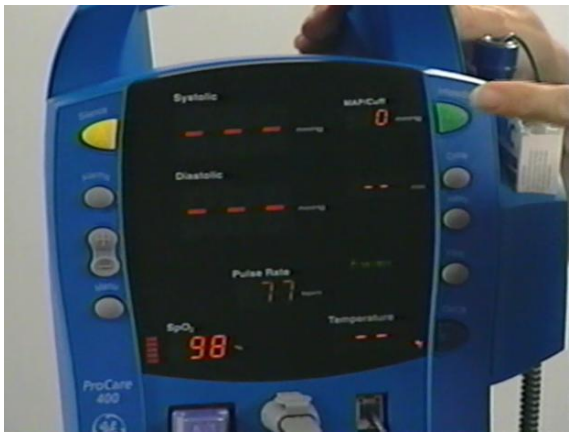

Figure 5

- Dynamap takes systolic and diastolic pressure, mean arterial pressure (MAP) and Pulse Rate of the Subject.
- Record the measurements in the case report form (CRF)
- Repeat the measurement second time and record in the CRF accordingly.
- Wait 25 to 30 seconds between each measurement.
- If either systolic or diastolic BP of the second measurement varies by more than 10mmHg, repeat the third measurement and record in CRF.
- FOR THE MOTHER - If the readings are abnormal i.e. >130/80mmHg, please ask the MOTHER to rest for 10 minutes before taking the 3rd measurement.