Investigating the impact of early-life, life-course and genetic factors on blood pressure among young Africans

Abubaker Swaib Lule

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Statement of own work

I, Abubaker Swaib Lule, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abubaker Swaib Lule

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Abstract

Background: The relationship between blood pressure (BP) and early-life factors such as birth weight, life-course factors such as obesity, and genetic variants among Africans are unknown. This PhD aimed to systematically review literature on the relationship between birth weight and BP among young Africans and to use data from the Entebbe Mother and Baby Study (EMaBS), a Ugandan birth cohort, to investigate the impact of early-life, life-course and genetic factors on adolescents' BP.

Methods: Four databases were systematically searched for relevant publications. In the EMaBS, data were collected prenatally from pregnant women and prospectively from the resulting offspring, with BP measured in adolescence. Linear regression was used to relate birth weight, early-growth and other characteristics to BP. Genetic analyses were conducted to investigate genetic variants associated with adolescents' BP.

Results: The systematic review showed that among the few published studies from Africa, the relationship between birth weight and BP varied by participants' age. Findings from the EMaBS indicated strong evidence of association between postnatal weight gain and later BP, with fast-growing low birth weight individuals particularly affected. Maternal factors positively associated with higher adolescent BP were gestational body mass index (BMI) and higher education status. Adolescent factors positively associated with higher BP were age, waist circumference, BMI, family history of hypertension and current *Trichuris* infection. Previous malaria infection in childhood and higher vegetable or fruit consumption were associated with lower BP in adolescence. No genetic variant reached genome-wide significance for association with BP. Thirty-three of 330 variants previously identified as associated with BP were replicated in this study, but none were significant after accounting for multiple testing.

Conclusions: Postnatal weight gain rather than birth weight is associated with later BP, with fast-growing low birth weight individuals at particular risk. Larger studies are required to characterise BP genetics in African adolescents.

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Thank you very much.

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List of acronyms

11β-HSD2	11β-hydroxysteroid dehydrogenase
ACEI	Angiotensin converting enzyme inhibitors
ARB	Angiotensin receptor blocker Antiretroviral
ART	therapy
BB	Beta-blockers
BCG	Bacille Calmette-Guerin
BMI	Body mass index
BMIZ	Body mass index for age Z-score
BP	Blood pressure
CCB	Calcium channel blockers
cm	Centimetres
CDC	Centers for Disease Control and Prevention
CEI	Converting enzyme inhibitors
COX2	Cyclooxygenase-2
CRH	Corticotropin releasing hormone
CRFs	Case report forms
CVDs	Cardiovascular diseases
DALYs	Disability-adjusted life-years
DNA	Deoxyribonucleic acid
DOHaD	Developmental origins of health and disease
ELISA	Enzyme-linked immunosorbent assay
EMaBS	Entebbe Mother and Baby Study
FFM	Fat free mass
FFMI	Fat free mass index
FM	Fat mass
FMI	Fat mass index
GCTA	Genome-wide complex trait analysis
GH	Growth hormone
GPS	Geographical positioning system
GWAS	Genome-wide association study
HAZ	Height-for-age Z-score
HICs	High-income countries
HMICs	High- and middle-income countries
IGF	Insulin-like growth factor
IHD	Ischaemic heart disease
IQR	Interquartile range

IUGR	Intrauterine growth retardation
LD	Linkage disequilibrium
LICs	Low-income countries
LR	Likelihood ratio
LSHTM	London School of Hygiene and Tropical Medicine
LVH	Left ventricular hypertrophy
MLP	Maternal low-protein
MR	Mendelian randomization
MRC	Medical Research Council
mRNA	Messenger ribonucleic acid
MUAC	Mid-upper arm circumference
NCDs	Non-communicable diseases
NHANES	National Health and Nutrition Examination Survey
PCR	Polymerase chain reaction
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analysis
PRISMA-P	Preferred Reporting Items for Systematic Review and Aeta-Analysis Protocols
RAS	Renin-angiotensin system
REC	Research and Ethics Committee
RT	Reverse transcription
SD	Standard deviation
s.e	Standard error
SGA	Small for gestational age
SNP	Single nucleotide polymorphism
SSA	Sub-Saharan Africa
STROBE	Strengthening The Reporting of Observational Studies in Epidemiology
TBW	Total body water
TBWI	Total body water index
UDHS	Uganda Demographic and Health Survey
UK	United Kingdom
UNCST	Uganda National Council of Science and Technology
UPHIA	Uganda Population-Based HIV Impact Assessment
USA	United States of America
UVRI	Uganda Virus Research Institute
WAZ	Weight-for-age *Z*-score
WHZ	Weight-for-height *Z*-score
WHO	World Health Organization

Chapter 1: Background

1.1 Introduction

This chapter provides background regarding the epidemiology of blood pressure (BP) and presents the hypothesis and the rationale for undertaking this PhD project. For the purpose of this chapter, high BP and hypertension are used interchangeably. High BP was defined as mean systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg in the adults (age ≥18 years) on one or more separate occasions or mean systolic and/or diastolic BP ≥95th percentile for sex, age and height based on readings taken on one or more separate occasions for children and adolescents (age 0-18 years). Pre-hypertension was defined as mean systolic BP between 120-139 mmHg and or diastolic BP between 80-89 mmHg in adults or mean systolic BP and/or diastolic BP ≥90th percentile but <95th percentile for sex, age, and height based on readings taken on one or more separate occasions in children and adolescents.

1.2 Blood pressure burden

Globally, high BP affects over a billion adults and contributes to over 10 million deaths annually [1-3]. In 2015, nearly 211 million disability-adjusted life-years (DALYs) were lost due to high BP [4]. High BP was the leading cause of global mortality and morbidity in 2013 [5]. The burden of high BP varies with geographical region and ethnicity, being highest in populations of African ancestries but lowest in Caucasians [6]. Approximately, 75% of the individuals with high BP are in low income countries (LICs) [7]. High BP is estimated to affect over 46% of adults above 25 years of age in Africa, compared to 35-40% in the same age group elsewhere in the world [8].

Diagnosis of cardiovascular diseases (CVDs) is uncommon before middle age. Yet important determinants, such as atherosclerosis and high BP, begin early in life [9]. Cardiovascular and metabolic changes begin long before clinical manifestation of disease [10]; left ventricular hypertrophy (LVH) occurs in more than a third of children with high BP [11] and fatty streaks were observed at autopsy of 15- to 19-year-old adolescents [12] and in aortas of premature fetuses [13].

The prevalence of CVDs determinants (including high BP, dyslipidaemia, diabetes mellitus and obesity) is high and rising in much of Sub-Saharan Africa (SSA) [14]. After allowing for differences in the age distribution of the populations, CVDs and related

determinants are affecting individuals at younger ages in SSA than in more affluent countries [15]. Once uncommon on the African continent, the burden of hypertension has grown continuously over the past decades. Despite a drop in the prevalence of high BP in high income countries (HICs) over the past four decades, LICs in SSA and Asia have seen an increase in the prevalence of high BP in the same period [16]. Earlier studies from Africa reported no or low burden of hypertension [17-19]. However, more recent studies from the continent summarised in systematic reviews have shown a high burden of hypertension in adults [20] and in young Africans [21]. The growing burden of high BP in Africa has been attributed to a transition from active to more sedentary lifestyles and a rise in unhealthy dietary practices (characterised by over consumption of saturated and trans fats) on the continent over the past decades [14, 16, 22-24].

Globally, the burden of hypertension has increased in children and adolescents. Between 1988 and 1999, prevalence of pre-hypertension and hypertension increased by 2% and 1% respectively, among American children [25]. In the early 2000s, the prevalence of hypertension doubled among South African adolescents [23]. In the United States of America (USA), the incidence rate of hypertension in adolescents was estimated at 7% per year [26]. Prevalence of high BP among young Africans varies between 1% and 25% [21, 27]. For example, it was estimated at 4% in Nigeria [28] and 4% in Uganda [29] based on readings on three different occasions and estimated at 8% in the Gambia [30], 10% in Seychelles [31], 17% in Uganda [29] and 21% in South Africa [32], based on the mean of 2 or 3 BP readings taken on the same occasion.

High BP is most commonly “essential hypertension” (no identifiable cause). Globally, essential hypertension affects 40-70% of children with hypertension [33, 34]. Essential hypertension is associated with obesity and family history of hypertension or CVDs [11]. Secondary hypertension (underlying cause present) is more common in children than adults and estimated to affect 30-60% of children with hypertension [33, 34].

Although high BP is less common in children than in adults, most adulthood hypertension initiates in childhood [35, 36]. A large proportion of those with childhood hypertension sustain the condition into adolescence [37, 38]. A study among 3,273 young black South Africans showed that children (mean age of 7.6 years at enrolment) maintained high BP into adolescence; after six years of follow-up, 36% of those with high BP remained hypertensive while 80% of the normotensives remained normotensive [38]. Among 1,927 Finnish participants between 3 and 18 years of age in 1980, observation of two or more

abnormal BP measurements was a good predictor of hypertension in these participants 21 to 31 years later [39].

Hypertension is a strong predictor of cardiovascular mortality in patients with end stage renal disease [40]; progression of chronic kidney disease [41]; and stroke in adults [42]. High BP in children is associated with CVDs markers such as LVH, atherosclerosis, intima media thickness, diastolic dysfunction and adulthood hypertension [11, 26, 43-46].

1.3 Diagnosis and treatment

1.3.1 Diagnosis

There is evidence that most hypertension is undetected and under treated. Globally, over 75% of hypertension in children and adolescents is estimated to be undiagnosed [47-49]. In HICs, only 13% to 26% of hypertension among children is properly diagnosed [33, 34]. Between November 2010 and October 2011, in a study of 29,000 children in the USA, identified as having three raised BP measurements at outpatient visits, only 215 (<1%) had been previously diagnosed as having high BP [49]. The burden of undiagnosed or inaccurately classified BP is undocumented among young Africans and could be higher than among children and adolescents from HICs.

Several challenges to hypertension diagnosis in children and adolescents exist. Blood pressure check-up (a vital medical examination) is routinely performed among adults but rarely in children or adolescents [47], despite the international recommendations to annually measure children's and adolescents' BP starting at three years of age in healthy individuals, and at every health care encounter if they are obese, or have renal disease, history of aortic arch obstruction or coarctation, or diabetes [11]. Unlike in adults for whom cut-offs for normal BP are clearly defined, in children and adolescents normal and abnormal BP values vary depending on sex, age, and height. This makes classification of BP in the young more challenging and cumbersome.

In addition, a cuff size appropriate for the child's arm (bladder length covering at least 80% of the mid-upper arm circumference (MUAC) but not more than 100% and bladder width covering at least 40% of the MUAC) is required for accurate measurement of BP [11]. Larger-than-appropriate cuff sizes give falsely low BP readings, a cuff that is too small for a child's arm will give a falsely high reading [50].

Blood pressure varies over time; thus, it is important to document raised BP from multiple readings for accurate diagnosis of high BP [11, 38, 51]. Following repeated BP measurements among 1,725 American adolescents (13 to 18 years), BP reduced to normal levels in 6% of those initially classified as having pre-hypertension (based on a single measurement) but increased to hypertensive levels in 3% of these initially classified as pre-hypertensive [51]. Pre-hypertensive children are at risk of developing hypertension later in life [52].

Hypertension in children is often asymptomatic and frequently missed by clinicians. Undiagnosed or untreated childhood hypertension is associated with serious health consequences, including organ damage (to organs such as the kidneys and heart) in childhood [11, 53, 54] and increased risk of hypertension [54], CVDs, stroke, and kidney disease in adulthood [42, 55]. Severe high BP is associated with increased risk of hypertensive encephalopathy, cerebrovascular accidents, myocardial infarction and congestive cardiac failure [53, 56]. Yet, treatment of BP reduces the risk for cardiovascular events [11] and could reverse and prevent the long-term sequelae to organ damage [48, 51, 53]. Therefore, identifying children and adolescents who already have, or who are at risk of developing, high BP is vital for the control and prevention of later cardiovascular events.

1.3.2 Treatment

Lifestyle modification (non-pharmacological) therapy is the first line treatment for primary hypertension in children [57, 58]. This consists of regular physical exercise, a diet restricted in salt (sodium) but rich in fresh fruits and vegetables, and reduction in weight for those who are overweight or obese [58, 59]. In addition to preventing the development of hypertension, regular physical exercise lowers BP levels among adults, with and without hypertension [60]. Moderate to vigorous physical exercise lasting 40 minutes for 3 to 5 days a week reduced systolic BP by 6-10 mmHg among 25 adolescents (aged 16 ± 1 years). However, BP levels returned to pre-training levels nine months later on cessation of physical exercises [61]. Interventions combining both dietary recommendations and regular physical exercise result in greater reductions in BP levels [60, 62].

Pharmacological therapy in children is recommended for symptomatic hypertension; secondary hypertension; persistent hypertension despite lifestyle modification therapy; hypertension with target-organ damage such as hypertensive retinopathy,

microalbuminuria, LVH; and presence of co-morbidities such as diabetes mellitus [63]. It is important to treat any underlying cause(s) of high BP.

Drugs from the angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blocker (ARB), beta-blockers (BB), calcium channel blockers (CCB) and diuretics are the first line agents for the treatment of paediatric hypertension [57]. Combination treatment with drugs from different classes is recommended if BP control cannot be attained with a single drug [59]. Pharmacological therapy aims at preventing organ damage and reducing long-term cardiovascular risk associated with hypertension [57]. Lifestyle modification remains the cornerstone therapy for primary prevention, treatment and control of hypertension and should be maintained when pharmacological treatment is initiated.

1.4 Blood pressure determinants

High BP is a complex multifaceted disorder; multiple environmental and genetic factors play a role in its development [64-66]. The modifiable determinants of BP include increased dietary salt intake, obesity, reduced physical activity, smoking, and poor sleep quality, while the non-modifiable determinants include gender, ethnicity, genetics and prematurity. Low birth weight and both low or high socio-economic status have also been associated with BP later in life.

1.4.1 Environmental (non-genetic) factors

Several environmental factors such as age [20, 30, 67-69], birth weight [70-75], gender [6, 32, 69, 76, 77], postnatal weight gain [74, 78-80], ethnicity [6, 81], waist circumference [32, 67, 82], urbanisation [20, 83, 84], socio-economic status [85, 86], physical inactivity [87, 88], sleep disorders and duration [11, 77], diet [88], dietary salt intake [89], family history of hypertension [90], body mass index (BMI) and obesity [21, 30, 32, 38, 67, 76, 82, 87] have been linked to BP, further details follow.

1.4.1.1 Blood pressure and early-life growth

1.4.1.1.1 Intra-uterine growth

Birth weight is not only an indicator of maternal gestational health and nutritional status but also an indicator of growth in utero and a newborn's chances of survival [91]. Low birth weight may be due to prematurity or intrauterine growth retardation (IUGR) or to being small for gestational age (SGA). The relationship between birth weight and BP later in life has been extensively studied, with several studies reporting an inverse association

between birth weight and BP [70-75]. Most of the studies on the relationship between birth weight and later BP come from high- and middle- incomes countries (HMICs).

As a component of this PhD project, a systematic review of studies on the relationship between birth weight and BP in young Africans was conducted. Details are presented in chapter 3. In brief the relationship between birth weight and BP was understudied, with 16 publications from 13 studies in nine African countries [92]. The studies were predominantly from South and West Africa and differed in size and design. The relationship between birth weight and BP varied with participants' age, with consistently positive associations seen in neonates (0-28 days), predominantly inverse associations in children (1-9 years) and a mixed picture (inverse or no association) in adolescents (10-19 years). A recent study (published after the review was submitted) among 528 rural South Africans aged 5 to 15 years, showed that birth weight was not associated with BP [93]. The relationship between birth weight and BP among African children and adolescents remains unknown and understudied [92].

Elsewhere, evidence from 444,000 individuals predominantly of non-African origin (aged 0 to 84 years) summarised in [74], showed an inverse relationship between birth weight and systolic BP. An inverse relationship between birth weight and systolic BP was reported among 66,000 participants aged 7 to 62 years from five European cohorts in three countries (United Kingdom [UK], Finland and Faroe Islands) reviewed in [94]. Restricting to studies in children and, or adolescents, evidence from 1,570 children aged 3 to 6 years from China, Guatemala, Chile, Nigeria and Sweden summarised in [95] showed that BP was inversely associated with size at birth except in Nigerian children. Among 5,600 children aged 3 to 6 years from China, birth weight was positively associated with BP [80]. Birth weight was not associated with BP among 250 American children and adolescents (1-14 years of age) of whom over 50% were blacks [96]. In 739 Brazilian children 6 to 10 years of age, birth weight was inversely associated with BP [97]. Among 405 British children aged 4 years from Salisbury, BP was inversely related to birth weight but positively associated with placenta weight [75].

Systematic reviews estimate that, overall, systolic BP drops by 2-4 mmHg for every one kg rise in birth weight [74, 98]. The effect of low birth weight on BP is thought to be due to fetal growth restriction rather than to prematurity. A study among 1,756 participants aged 34-49 years from Finland reported that BP was 7.2 mmHg higher among adults who were small for gestational age at birth compared to adults who were preterm with

appropriate weight for gestational age and 7.3 mmHg higher compared to those who were term and of normal birth weight [99].

On average, earlier publications have shown an inverse association between birth weight and later BP, but the more recent studies cast doubt on this inverse relationship, indicating that the relationship between birth weight and BP could be "U-shaped": both low and high birth weight are associated with high BP. From 31 studies reviewed in [100], individuals with high birth weight (≥4,000g) compared to those with normal birth weight (2,500-4,000 g or the 10-90th percentiles for gestational age) had increased BP in childhood. In the Netherlands, low and high birth weight were associated with high BP in 392 children aged four years [101]. In the UK, higher maternal intake of carbohydrates and proteins in pregnancy was associated with hypertension in the resulting 253 offspring, 40 years later [102].

There may also be a role for effect modifiers of the association between birth weight and BP. In a study of 959 15-year-old British adolescents, the relationship between birth weight and BP differed by gender: a negative association among males and a positive association in females [103]. Studies from the USA have shown racial disparities in the relationship between birth weight and BP later in life [104, 105]. A study among 29,710 mothers and their seven-year-old offspring, reported a positive association between birth weight and BP among black children and no association among white children [104]. In another study among 262 American children aged five years, systolic BP decreased by 6 mmHg per kg increase in birth weight in white children and increased by 2 mmHg per kg increase in birth weight in African American children [105].

The relationship between BP and other measures of birth size including birth length, head circumference, placenta size and ponderal index has also been studied. Occipito-frontal circumference and head circumference but not length at birth, were inversely associated with BP among 756 Zimbabwean school children (average age of 6.5 years) from Harare [106]. Except for birth weight which was positively associated with BP, other birth parameters (head circumference and length) were not associated with BP in 634 newborn healthy Brazilians [107]. A previous systematic review of more than 444,000 participants aged 0-84 years, estimated that systolic BP decreases by 0.5 mmHg per centimetre (cm) increase in head circumference [74]. Both ponderal index and birth length were inversely associated with systolic BP among 122 participants from China followed from birth to age 30 years: systolic BP decreased by 1.8 mmHg per unit increase in ponderal index and by 3.2 mmHg per cm increase in birth length [108]. Head circumference or birth length

or ponderal index were not associated with BP among 1,860 English children aged three years [109].

Among 449 British adults aged 46 to 54 years, systolic BP was estimated to increase by 15 mmHg when placental weight increased from ≤1 lb to >1.5 lb with the highest increase among those who were small at birth but had large placentas [73]. Among 13,273 Americans who were full-term singletons at birth, placental weight was positively associated with systolic BP at seven years of age but there were no associations between placental weight and systolic BP at four months of age [110]. Gestational age was inversely related to BP among 5-7 year olds from the UK [111]. Gestational age was inversely correlated with BP among 430 Swedish males aged 49 years, born between 1926 and 1927, with strong negative correlation (r= -0.46) in preterm (born before 38 weeks of gestation) but no correlation between BP and gestational age in those born after 38 weeks of gestation [112].

In summary, the weight of evidence suggests that birth weight is inversely related to BP later in life. However, current literature suggests that the relationship between birth weight and later BP could be U-shaped. The relationship between birth weight and BP later in life remains understudied among populations from SSA. Possible effect modifiers (such as season of birth or conception and sex) of the relationship between birth weight and BP later in life need further investigation.

1.4.1.1.2 Postnatal growth

Enhanced postnatal growth (measured as greater than expected weight gain soon after birth) in early childhood has been associated with higher BP later in life [74, 78-80].

Studies (predominantly from Europe, none from Africa) published between 1996 and 2000 on individuals aged 3 to 26 years, reviewed in [74] reported positive associations between increased postnatal growth (weight gain or change in BMI) [from birth to current size] and systolic BP. Another study among 15,600 3- to 6-year-old Chinese children (published in 2011) reported a positive relationship between postnatal weight gain and hypertension later in life [80]. Individuals who experience IUGR accompanied by accelerated postnatal weight gain ("nutritional mismatch") may be at an increased risk for high BP later in life than their counterparts who do not experience accelerated postnatal weight gain [78].

Among 530 children from Massachusetts, accelerated weight gain in the first six months of life was positively associated with systolic BP at three years of age [79]. Rapid weight

gain between one and five years of age was associated with the highest increase in BP among 22-year-old British adults who were small at birth [113]. In the Seychelles, changes in weight during successive periods since birth contributed to high BP among children and adolescents, with stronger effects of increased growth in later childhood than in infancy and early childhood [114]. The critical growth period(s) which may lead to an effect on later BP remains unclear.

Globally, increased postnatal weight gain in the first years of life is associated with later high BP but this relationship differs by size at birth. The effect of increased postnatal weight gain on later BP is understudied and remains unknown in individuals from SSA.

1.4.1.2 Other environmental determinants

Blood pressure increases with age [68, 69]. A study in the USA among 730 infants, showed that average systolic BP increased from 70 mmHg at birth to 85 mmHg at one month of age; to 92 mmHg at six months of age; and to 95 mmHg at one year of age [68]. Population based studies (each with over 400 participants above the age of 15 years) reviewed in [20], showed that the proportion of individuals with high BP increased with increasing age.

On average men have higher BP compared to age-matched women before menopause [115]. High BP is more common in male children and adolescents than in their female contemporaries [32, 76, 77]. Based on an average of three BP readings at each screening in 5,102 children from Texas, USA, the prevalence of high BP was 23% in males but 16% in females at first screening and was 6% in males and 3% in females at the third screening [76]. Among 1,771 adolescents aged 13 years from Portugal, 35% of males compared to 30% of females had high BP [77].

Over 50 studies among adults, predominantly from middle and high income countries, published by March 2014 and reviewed in [85], showed that low socio-economic indicators (income, occupation category or level of educational) were each associated with increased risk of hypertension. Among 1,008 Nigerian adolescents aged 10 to 17 years, reduced maternal socio-economic status was associated with increased BP [86]. Yet among 410 Brazilian students (mean age of 16 years), there was no association between socio-economic factors and BP [116].

Urbanisation is another socioeconomic indicator associated with high BP. High BP is more prevalent in urban areas than in rural areas [20, 83, 84]. In a systematic review involving studies from Africa published between January 1975 and May 2006, prevalence

of hypertension was higher in urban populations compared to rural populations in nearly all studies that included both urban and rural populations [20]. A multinational study of 1,216 participants from four sub-Saharan countries of Nigeria, South Africa, Tanzania and Uganda reported that the prevalence of both hypertension and pre-hypertension differed by occupation and level of urbanisation. The age-standardized hypertension prevalence was 26% among nurses compared to 23% in school teachers and 21% in peri-urban residents compared to 9% in rural residents [84]. But in a nationwide survey conducted from March to July 2014 among 3,906 Ugandans aged 18 to 69 years, prevalence of hypertension was similar in urban and rural populations at 29% and 26%, respectively [83]. Any differences in prevalence between urban and rural areas may be due to differences in lifestyle and or infection intensity. Urban areas have higher proportions of obese residents, increased daily salt and fat intake (consumed from processed and fast foods) and reduced physical activities due to the sedentary lifestyle compared to rural residents [20, 117].

In Kenya, sickle-cell trait (linked with partial protection against malaria) was associated with lower BP in Kilifi (currently a low-moderate but historically a high malaria transmission area) but not in Nairobi (no malaria transmission), suggesting a positive association between malaria and BP [118]. In rural Ghana, malaria in pregnant women at delivery was associated with increased systolic BP in the resulting 155 adolescents (mean age of 15 years [119].

Among American children, unhealthy food choices (increased sugar-sweetened drinks) were associated with increased systolic BP [88, 120]. A nutritional survey in 4,867 adolescents aged 12 to 18 years from the USA reported that higher intake of sugar-sweetened beverages was associated with increased systolic BP [120]. Fruit and vegetable intake were inversely associated with BP among 163 16- to 17-year-old Americans [121]. Increased sodium intake was associated with high BP in a study of 6,235 Americans aged 8 to 18 years, with the strongest relationship in overweight or obese individuals [122]. Increased dietary salt intake is linked with raised BP [123]. Sources of dietary sodium are mainly manufactured foods and salt added to cooked food at the table [89].

Obese children are three times more likely to develop high BP than their non-obese colleagues [124]. Studies summarised in [82], estimated prevalence of hypertension at 11-30% among obese children and 3-14% in normal weight children [82]. Studies published from 1st January 1996 to 2nd February 2017 on 54,196 African children and adolescents aged between 2 and 19 years reviewed in [21] reported that the prevalence of

high BP was six times higher in obese individuals compared to normal-weight participants. High BMI was associated with high BP among 201 Ghanaian youths 15 to 25 years of age from Accra [87]. Obesity accounted for nearly 20% of the high BP among 15,612 children aged 5-16 years from the Seychelles [31].

Exposure to maternal cigarette smoking (active and or passive smoking) during pregnancy was associated with high BP levels in the offspring born in Patras, Greece, with a dose-response effect related to the number of cigarettes smoked per day [125].

Reduced physical activity was associated with increased BP in 201 Ghanaian youths aged 15-24 years [87] and 67 Chinese American children with a mean age of 9 years [88]. A study among 345 school children, showed that physical activity levels decline with age, this decline in physical activity with age was more rapid in females than in males [126].

Poor sleep quality was associated with pre-hypertension in 238 healthy adolescents aged 13 to 16 years in the USA [127]. Short sleep duration and increased television viewing were associated with high BP among 4,452 Brazilian adolescents aged 10-12 years [128]. However, among 1,771 13-year-old Portuguese adolescents, sleep duration was positively associated with BP in females but not among males [77]. Maternal history of high BP but not paternal history of high BP was associated with high BP among 3,360 children of European origin aged 5-7 years from England and Wales [111].

1.4.2 Genetic determinants

Besides environmental influences, genetic factors play important roles in the development of high BP [64]. Children with a hypertensive parent are at increased risk of developing hypertension [129]. Studies estimate heritability of BP between 15-35% [64, 65, 129]. Heritability of systolic BP was estimated at 30% among 8,451 Americans aged 45 to 64 at enrollment in the Atherosclerosis Risk in Communities study [130]. African ancestry is associated with hypertension [6, 131-133].

Among UK adolescents, after adjusting for anthropometry and social exposures, systolic BP at age 16 years was 2.9 mmHg greater, on average, in black adolescents compared to white adolescents [133]. This excess burden of high BP among blacks may be due to increased genetic susceptibility to high BP in individuals of African ancestry.

Genetic studies of BP have the potential to enhance our understanding and treatment of high BP. Genome-wide association studies (GWAS) conducted using participants from HICs have identified several single nucleotide polymorphisms (SNPs) associated with both systolic and diastolic BP [134, 135]. SNPs in genes such as *ATP2B1, MTHFR,*

YP17A1, FGF5, CSK and *STK39* have been linked with hypertension in Caucasian populations [135-138], while in African Americans, variants in the *C21ORF91* and *GPR98/ARRDC3* genes have been associated with systolic and diastolic BP, respectively [139].

The *ATP1B1* gene encodes for the β subunit of Na,K-ATPase, a protein for maintaining electrochemical gradients of Na^+ and K^+ across the plasma membrane [140]. Reduced Na,K-ATPase activity preceded development of hypertension in experimental rats [141]. The *MTHFR* gene encodes for methylenetetrahydrofolate reductase which metabolises folate. Reduced folate levels result in hyperhomocysteinemia, a condition associated with hypertension [142]. The *STK39* gene encodes for SPAK protein, a protein involved with salt transport (renal salt excretion) and osmotic cell volume regulation [143].

Replication studies have returned variable results in different populations [135, 144] and this lack of consistency across studies weakens any generalisable conclusions. The BP GWAS meta-analysis of 29, 378 African Americans identified only the *SOX6* locus of the five loci that were identified to be associated with BP in a multi-ethnic (African American, Europeans and East Asian) sample of 99,382 individuals [145]. Of the 17 SNPs most strongly associated with BP (p-value $<1x10^{-4}$) in African Americans, three SNPS (rs1867226, rs1550576 and rs8039294) were replicated (p-value <0.05) in a sample of West Africans [146]. Loci identified in Caucasian and Asian populations are yet to be replicated in African populations [145, 146]. From twin studies, 30% of BP variability was due to heritability [64, 65] but, the SNPs associated with BP account for only 2-5% of BP variation [147, 148]. Thus, important undiscovered variants associated with BP are likely to exist. This "missing heritability" may be due to rare varaints or possibly due to combinations of common variants with small effect sizes [149, 150].

In summary, high BP is a complex disorder influenced by a combination of environmental and genetic factors. Prevalence of high BP remains high world-wide and steadily increasing in certain populations. In children and adolescents, most high BP is undiagnosed or inaccurately diagnosed. Several environmental and genetic factors are associated with high BP.

1.5 Developmental origins of health and disease hypothesis

The developmental origins of health and disease (DOHaD) hypothesis proposes that early-life environments (periconceptual, fetal and early postnatal) have long-lasting influences in offspring to later health and disease susceptibility.

Poor maternal nutrition during pregnancy contributes to restricted fetal growth, leading to increased susceptibility to high BP later in life.

We postulated that growth in early-life (utero and or childhood) is associated with high BP later in life (an inverse association with birth weight and a positive association with early postnatal growth) and that several environmental and genetic factors (more commonly present in Africa than in other settings) are associated with BP in young Africans.

1.5.1 Evidence from epidemiological studies

Initial work on the relationship between fetal environment and later cardiometabolic diseases was conducted by David Barker and colleagues at the University of Southampton, in the UK. They showed strong geographical correlation between infant mortality that occurred between 1921 and 1925 and adult mortality from ischaemic heart disease (IHD) occurring from 1968 to 1978 in England and Wales [151]. It was postulated that factors that increased the risk of death during infancy also increased susceptibility to IHD among those who survived through infancy. The group later showed an inverse association between birth weight and BP and a positive relationship between placenta size and BP among adults (46 to 54 years) from Preston, Lancashire [73].

Subsequently, these findings have been replicated world-wide [70, 71, 152-154], with birth weight the most studied birth parameter. Birth weight is an important marker of fetal nutrition and growth [91]. Poor maternal nutrition in pregnancy results in low birth weight in the newborn, while maternal overnutrition during pregnancy leads to high birth weight in newborns [155, 156].

Evidence from the Dutch Hunger Winter Study comprising of 2,414 individuals, who were term and singleton, born around the Dutch famine (between November 1943 and February 1947), suggests that maternal undernutrition during gestation had important effects on health in later life for example, those who were small at birth had high BP later in life [157, 158]. The effects on health depended on the type and timing of insult or stimulus during gestation [159], for example offspring of mothers who consumed relatively little protein in relation to carbohydrate in the third trimester of pregnancy had high BP in adult life [158].

Epigenetic changes from prenatal malnutrition also influence metabolic phenotypes in later life. Epigenetic modulation of pathways by prenatal malnutrition may promote an adverse metabolic phenotype in later life [160]. The Swedish Twin Study showed that the

shorter twin in a twin pair was more likely to die of heart disease than their taller twin sibling [161].

Overall, epidemiological studies have provided convincing support for the DOHaD hypothesis by showing strong associations between low and or high birth weight and subsequent risk of adulthood hypertension.

1.5.1.1 Fetal growth

Earlier studies have mainly reported inverse relationships between birth weight and later BP. However, recent studies cast a doubt on the inverse relationship between birth weight and later BP, suggesting that both low birth weight and high birth weight may be important in the development programming of high BP later in life [70-74, 80, 97, 101]. This U-shaped relationship between birth weight and later high BP highlights the importance of both fetal under- and over-nutrition in the developmental programming of later BP.

1.5.1.2 Postnatal growth

Recent evidence suggests that postnatal growth rather than or in addition to fetal growth is important in the developmental programming of BP, with the fast-growing low birth weight offspring at a particularly increased risk for high BP later in life. Increased change in weight (a proxy for rapid growth in early childhood) resulting from excess postnatal nutrition is associated with higher BP later in life [74, 79, 80].

Early-life nutritional deprivation resulting in lower birth weight and or IUGR accompanied by later nutritional excesses (rapid postnatal weight gain), resets homeostatic systems (such as the endocrine and metabolic) in these offspring [78, 162]. Offspring who experience “a nutritional mismatch” between fetal life (slow growth in utero) and postnatal life (accelerated postnatal growth) are at an increased risk for later high BP [78]. This demonstrates the importance of the “nutritional mismatch” between the predicted and realised future in the developmental programming of later diseases.

1.5.2 Evidence from animal studies

Animal models have provided the strongest insight into potential DOHaD mechanisms [163-166], further details are covered later in section 1.6. Early under- or over-nutrition resulting from maternal dietary restriction or impaired placental perfusion are associated with hypertension in adulthood [167, 168]. Rats fed on a restricted diet from conception through pregnancy or lactation had hypertensive adult offspring. Maternal or fetal undernutrition are linked with changes in offspring cardiovascular function [169].

1.6 Programming mechanisms

The observed phenomena are not fully explained by a single mechanism but could be due to interactions between various mechanistic processes. Permanent structural and functional changes

It has been shown that early-life insults or stimuli (such as nutrition or hormones) occurring during critical growth periods (periods of rapid development) result in survival responses with long term consequences on health and disease susceptibility [170]. In humans and in animal models, maternal undernutrition greatly impacts a rapidly growing fetus through loss in structural units such as nephrons resulting in changes in organ growth, structure, function and metabolism in the resulting offspring [164, 170, 171].

During suboptimal nutritional or hormonal levels, the fetus' immediate aim to survive and preserve vital functions occurs at the expense of other less critical functions [172]. Diverting blood supply away from these organs (such as the kidneys) alters their normal development and results in changes in structure (to small organs) and function (hormonal and metabolic) [173-175]. Although, these adaptations are advantageous for short-term survival, they can be detrimental for health in the long-term.

Animal models have shown that early-life environmental conditions can have permanent effects on kidney structure and function. A suboptimal early environment leads to a reduction in nephron numbers via a reduction in nephrogenesis [176] or increase in apoptosis thus leading to a reduction in renal progenitor cells [177]. Placental insufficiency in rats was associated with increase in renal apoptosis in IUGR offspring [178]. Nutritional restriction in sheet [179] and rats [180, 181] and placental insufficiency in rabbits [182] and rats [183] resulted in reduced number of nephron in the offspring.

The loss of nephrons in a developing kidney programs the growing organ(s) to a reduced functional capacity. These small kidneys with a reduced number of functional nephrons maintain haemodynamic and excretory functions through increasing local vascular resistance, increasing local kidney BP [164]. This gradually leads to loss of more functional nephrons and further increases local BP and subsequently increases systemic BP. Intrauterine growth retardation (measured as low birth weight) is often due to an adverse intrauterine environment such as nutritional deprivation and associated with high BP later in life [70-73]. At post-mortem, IUGR was associated with reduced nephron numbers among human stillborn infants [184]. The impact of low birth weight and of

IUGR on kidney function may be mediated through a reduction in the number of functional nephrons [185, 186].

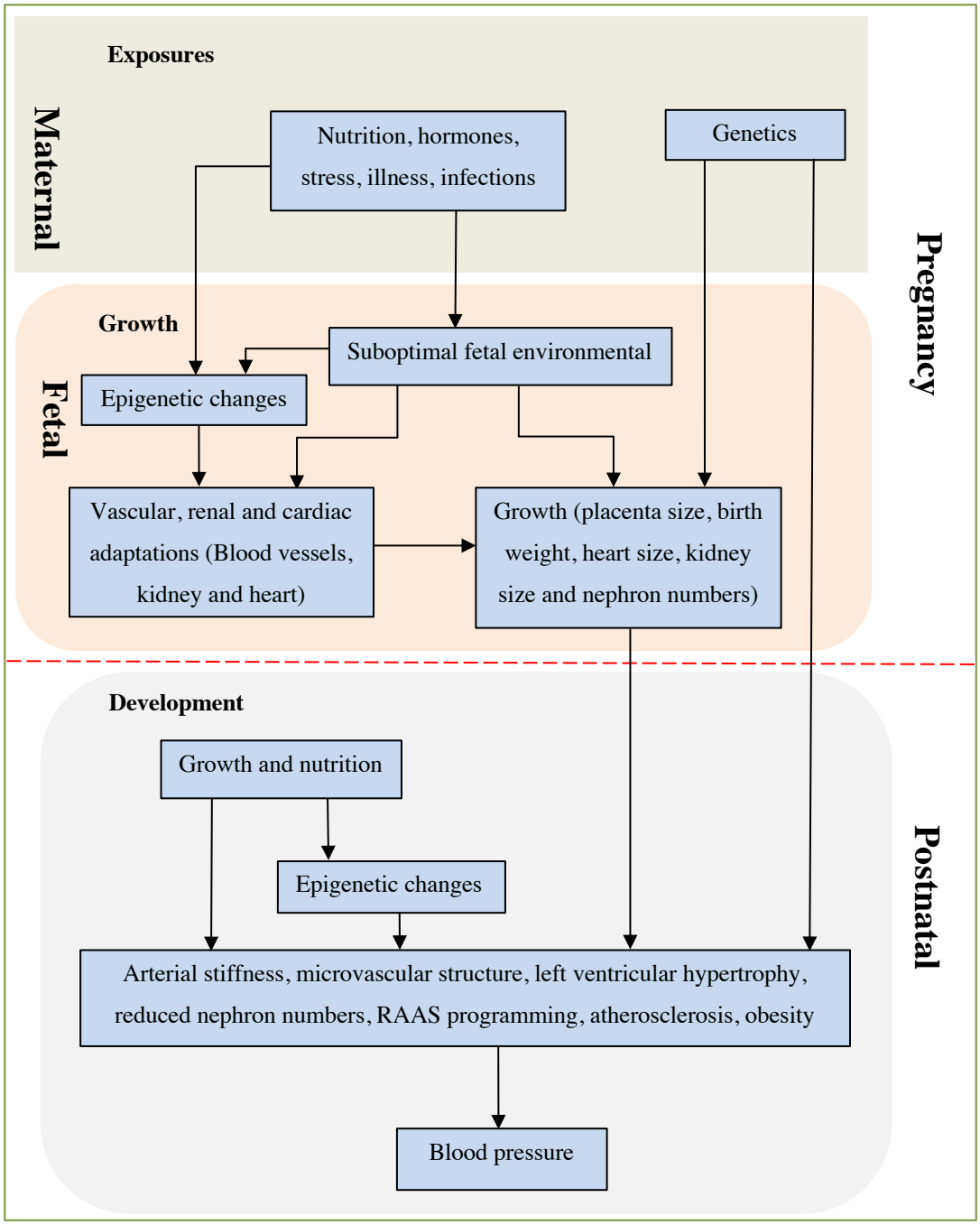

Figure 1.6-1:Mechanisms for the developmental programming of blood pressure model

The decrease in nephron endowment is also thought to be secondary to changes in regulatory mechanisms such as renin-angiotensin system (RAS) or cyclooxygenase-2 (COX2) activity. In animal models, administration of steroids or low protein intake in pregnancy or reduction in uterine perfusion induces a decrease in RAS activity and thus decreased nephron endowment [187, 188]. Administration of converting enzyme

inhibitors (CEI) or AT1 receptor antagonists (ARA) during kidney development result in low birth weight and reduced nephron endowment. The role of RAS in the developmental programming of BP is supported by studies that have shown that administration of CEI or AT1 receptor antagonist (ARA) decreases BP [170]. Reductions in Angiotensin II in the nephrogenic periods modify kidney function and induce sodium sensitivity during aging resulting in angiotensin II–dependent hypertension [189]. Higher renal sensitivity to Angiotensin II is associated with renal vasoconstriction [170].

Changes in circulation dynamics and organ growth during suboptimal conditions are likely to be hormonally-mediated. The glucocorticoids influence cellular differentiation and growth. The 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme metabolises cortical and corticosterone (glucocorticoids) into inert products, reducing amount of maternal glucocorticoid available to the developing fetus, thus, protecting the fetus from the effect of exposure to excess maternal glucocorticoids [190]. Maternal undernutrition reduces placental 11β-HSD2 increasing the quantity of glucocorticoids available to the fetus via placenta transfer [167, 173].

Exposure to excess maternal glucocorticoid during pregnancy retards fetal growth and is associated with low birth weight and with hypertension in the offspring [167]. The role of glucocorticoids in developmental programming of BP is supported by human studies that have shown associations between low birth weight and increased cortisol levels [173, 191]. Rats and ewes exposed to dexamethasone in pregnancy gave birth to offspring with reduced nephron numbers and high BP [165, 166]. The offspring of rats fed on maternal low-protein (MLP) diet and treated with metyrapone (an inhibitor of maternal glucocorticoid synthesis) did not develop high BP [164]. In contrast, pregnant rats fed on MLP diet and received corticosterone replacement had hypertensive offspring [164].

Maternal anaemia results in poor placental vascularization and deregulation of maternal and fetal hormones (including corticotropin-releasing hormone (CRH) and Insulin-like growth factor (IGF1), which stimulates fetal growth) [192]. The release of CRH induces preterm labour and pre-eclampsia in women and subsequently inhibits the production of IGF1 [192]. In the fetus, growth hormone (GH)-IGF-I axis is affected by maternal nutrient restriction [186]. Deficiency of GH was associated with increased hypertension among men [193]. Also, IGF1 was reduced both in pregnancies that resulted in growth retarded neonates and in the cord blood of IUGR neonates [170]. In rodents, maternal iron restriction resulted in low birth weight and high BP in the offspring [194, 195]. Reduced or poor placental perfusion due to abnormal placental vascularization from

preeclampsia often results in IUGR. Placental insufficiency induced via reduction in uterine perfusion in pregnant rats resulted in IUGR offspring with increased risk for hypertension [163].

1.6.1 Epigenetic changes

Changes in the early environment result in permanent changes in gene expression following multiple rounds of cell division [196]. Epigenetic changes (in deoxyribonucleic acid (DNA) methylations, histone acetylation and transcriptional activation) alter gene expression without changing the genome (DNA sequences) [197]. It has been suggested that maternal pregnancy protein restriction may result in hypertensive offspring by altering DNA methylation and gene expression [162].

In sheep, DNA methylation was associated with high BP in adult sheep [198]. While in rats, DNA methylations resulted in changes in glucocorticoid receptor in offspring rats [199]. Prenatal treatment of rats with dexamethasone resulted in increased BP and increased histone deacetylase1 in the kidneys accompanied by increased angiotensin II AT1 receptor in the offspring. Yet treatment with L-citrulline in pregnancy and lactation reversed these molecular changes and prevented hypertension in the offspring [200].

1.6.2 Genetics

Genetic mechanisms for the developmental origins of BP are unclear. It is suggested that genetic variants/single-nucleotide polymorphisms (SNPs) influence disease by altering the gene function or gene expression or the encoded protein [136, 201]. Previous GWAS of BP have identified several SNPs associated with BP, in populations from HMICs [134-139]. The rs17249754 variant in the *ATP2B1* gene is one of the SNPS associated with BP [135]. The *ATP2B1* gene, highly expressed in the vascular endothelium regulates cellular calcium levels that are important in vascular smooth muscles contraction and dilatation [202]. Also, higher levels of *ATP2B1* messenger ribonucleic acid (mRNA) were detected in smooth muscle cells of hypertensive rats compared with non-hypertensive controls [203].

1.7 Study rationale

Once uncommon on the African continent, prevalence of CVDs has increased and affects younger ages than in HICs [14, 15, 17, 19]. The African region has also seen an upsurge in CVDs determinants such as high BP, obesity, dyslipidaemia and diabetes mellitus [14]. The rising burden of these conditions in Africa has been attributed to a transition from

active to more sedentary lifestyles and a rise in unhealthy dietary practices [14, 16, 22], characterised by over consumption of saturated and trans fats [24].

To date, most literature supporting the DOHaD hypothesis come from HMICs. However, the hypothesis may be pertinent to Africa, a continent which continues to suffer a high burden of malnutrition (both maternal and infant) and a rapidly increasing burden of overnutrition (obesity) and CVDs and related determinants [14, 204-206].

Findings from Caucasian populations may not be applicable to African populations and cannot be extrapolated to LICs. There are differences in prevalence of environmental (lifestyle, infections and infection intensity) and genetic factors between rural, tropical Africa, developed or sub-tropical settings and HICs. For example, in HICs, prematurity, which can be due to any one of various factors (and their combination), including obstetric conditions, maternal BMI, maternal smoking, maternal alcohol consumption and environmental exposures, is the leading cause of low birth weight [196, 207, 208]. Yet in developing countries, IUGR (often resulting from maternal malnutrition and infections such as HIV and malaria, more prevalent in the tropics) accounts for many low birth weight infants [209, 210]. Other causes of low birth weight such as maternal alcohol and smoking are less common in Africa, than in some other settings in HMICs.

The burden of malnutrition remains high in Africa [204-206]. A combination of fetal undernutrition and rapid postnatal growth may accelerate the rising burden of BP and other CVDs risk factors in Africa. High BP initiates early in life, persists into adulthood [37, 38] and predicts adulthood hypertension [11, 46]. Children and adolescents are important target groups for high BP prevention and control interventions before clinical manifestation of BP and, or CVDs.

Despite African populations having the highest burden of high BP [20, 21], high genetic diversity [211] and low linkage disequilibrium (LD) [tendency for groups of genes to be inherited together] [212], genetic factors associated with BP in adolescents are unknown and remain understudied on the continent. In addition, genetic studies in Caucasians have identified several genetic variants associated with BP, but these findings are yet to be replicated in individuals residing on the African continent [145, 146].

The impact of early-life, life-course and genetic factors on later BP among young Africans remains unknown and understudied. This PhD aims to describe early-life, life-course and genetic factors associated with BP among young Africans. Identifying early-

life factors associated with BP later in life among Africans has huge potential to inform policy and mitigate the escalating burden of high BP and related CVDs in the region.

1.8 References

1. Poulter, N.R., D. Prabhakaran, and M. Caulfield, Hypertension. Lancet, 2015. 386(9995): p. 801-12.
2. Lim, S.S., et al., A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet, 2012. 380(9859): p. 2224-60.
3. WHO, A global brief on hypertension: silent killer, global public health crisis. 2013, World Health Organization Press: Geneva.
4. Collaborators, G.B.D.R.F., Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet, 2016. 388(10053): p. 1659-1724.
5. Collaborators, G.B.D.R.F., et al., Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet, 2015. 386(10010): p. 2287-323.
6. Hertz, R.P., et al., Racial disparities in hypertension prevalence, awareness, and management. Arch Intern Med, 2005. 165(18): p. 2098-104.
7. Mills, K.T., et al., Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries. Circulation, 2016. 134(6): p. 441-50.
8. Dzudie, A., et al., Roadmap to achieve 25% hypertension control in Africa by 2025. Cardiovasc J Afr, 2017. 28(4): p. 262-272.
9. Expert Panel on Integrated Guidelines for Cardiovascular, H., et al., Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. Pediatrics, 2011. 128 Suppl 5: p. S213-56.
10. Hardy, R., D.A. Lawlor, and D. Kuh, A life course approach to cardiovascular aging. Future Cardiol, 2015. 11(1): p. 101-13.

11. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics, 2004. 114(2 Suppl 4th Report): p. 555-76.

12. Strong, J.P., et al., Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. JAMA, 1999. 281(8): p. 727-35.

13. Palinski, W. and C. Napoli, The fetal origins of atherosclerosis: maternal hypercholesterolemia, and cholesterol-lowering or antioxidant treatment during pregnancy influence in utero programming and postnatal susceptibility to atherogenesis. FASEB J, 2002. 16(11): p. 1348-60.

14. Dalal, S., et al., Non-communicable diseases in sub-Saharan Africa: what we know now. Int J Epidemiol, 2011. 40(4): p. 885-901.

15. Lopez, A.D., et al., Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet, 2006. 367(9524): p. 1747-57.

16. Collaboration, N.C.D.R.F., Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19.1 million participants. Lancet, 2017. 389(10064): p. 37-55.

17. Pobee, J.O., et al., Blood pressure distribution in a rural Ghanaian population. Trans R Soc Trop Med Hyg, 1977. 71(1): p. 66-72.

18. Shaper, A.G. and G.A. Saxton, Blood pressure and body build in a rural community in Uganda. East Afr Med J, 1969. 46(5): p. 228-45.

19. Donnison, C.P., Pressure in the African Native: its bearing upon the aetiology of hyperpiesia and arteriosclerosis. The Lancet, 1929. 213(5497): p. 6-7.

20. Addo, J., L. Smeeth, and D.A. Leon, Hypertension in sub-saharan Africa: a systematic review. Hypertension, 2007. 50(6): p. 1012-8.

21. Noubiap, J.J., et al., Prevalence of elevated blood pressure in children and adolescents in Africa: a systematic review and meta-analysis. The Lancet Public Health, 2017. 2(8): p. e375-e386.

22. Bosu, W.K., An overview of the nutrition transition in West Africa: implications for non-communicable diseases. Proc Nutr Soc, 2015. 74(4): p. 466-77.

23. Mangena, P., et al., An approach to the young hypertensive patient. S Afr Med J, 2016. 106(1): p. 36-8.

24. Ginter, E. and V. Simko, New data on harmful effects of trans-fatty acids. Bratisl Lek Listy, 2016. 117(5): p. 251-3.

25. Din-Dzietham, R., et al., High blood pressure trends in children and adolescents in national surveys, 1963 to 2002. Circulation, 2007. 116(13): p. 1488-96.

26. Falkner, B., Hypertension in children and adolescents: epidemiology and natural history. Pediatr Nephrol, 2010. 25(7): p. 1219-24.

27. Monyeki, K. and H. Kemper, The risk factors for elevated blood pressure and how to address cardiovascular risk factors: a review in paediatric populations. J Hum Hypertens, 2008. 22(7): p. 450-9.

28. Okpokowuruk, F.S., M.U. Akpan, and E.E. Ikpeme, Prevalence of hypertension and prehypertension among children and adolescents in a semi-urban area of Uyo Metropolis, Nigeria. Pan Afr Med J, 2017. 28: p. 303.

29. Kidy, F., et al., Blood pressure in primary school children in Uganda: a cross-sectional survey. BMC Public Health, 2014. 14: p. 1223.

30. Jobe, M., et al., High blood pressure and associated risk factors as indicator of preclinical hypertension in rural West Africa: A focus on children and adolescents in The Gambia. Medicine (Baltimore), 2017. 96(13): p. e6170.

31. Chiolero, A., et al., Prevalence of elevated blood pressure and association with overweight in children of a rapidly developing country. J Hum Hypertens, 2007. 21(2): p. 120-7.

32. Nkeh-Chungag, B.N., et al., Prevalence of hypertension and pre-hypertension in 13-17 year old adolescents living in Mthatha - South Africa: a cross-sectional study. Cent Eur J Public Health, 2015. 23(1): p. 59-64.

33. Report of the Second Task Force on Blood Pressure Control in Children--1987. Task Force on Blood Pressure Control in Children. National Heart, Lung, and Blood Institute, Bethesda, Maryland. Pediatrics, 1987. 79(1): p. 1-25.

34. Update on the 1987 Task Force Report on High Blood Pressure in Children and Adolescents: a working group report from the National High Blood Pressure Education Program. National High Blood Pressure Education Program Working

Group on Hypertension Control in Children and Adolescents. Pediatrics, 1996. 98(4 Pt 1): p. 649-58.

35. Flynn, J.T., Pediatric hypertension update. Curr Opin Nephrol Hypertens, 2010. 19(3): p. 292-7.

36. Sadoh, W.E., et al., Predictors of newborn systolic blood pressure. West African Journal of Medicine, 2010. 29(2): p. 86-90.

37. Kagura, J., et al., Blood pressure tracking in urban black South African children: birth to twenty cohort. BMC Pediatr, 2015. 15: p. 78.

38. Miersch, A., et al., Blood pressure tracking in children and adolescents. Pediatr Nephrol, 2013. 28(12): p. 2351-9.

39. Oikonen, M., et al., Repeated Blood Pressure Measurements in Childhood in Prediction of Hypertension in Adulthood. Hypertension, 2016. 67(1): p. 41-7.

40. Groothoff, J.W., et al., Mortality and causes of death of end-stage renal disease in children: a Dutch cohort study. Kidney Int, 2002. 61(2): p. 621-9.

41. Wingen, A.M., et al., Randomised multicentre study of a low-protein diet on the progression of chronic renal failure in children. European Study Group of Nutritional Treatment of Chronic Renal Failure in Childhood. Lancet, 1997. 349(9059): p. 1117-23.

42. Leiba, A., et al., Hypertension in late adolescence and cardiovascular mortality in midlife: a cohort study of 2.3 million 16- to 19-year-old examinees. Pediatr Nephrol, 2016. 31(3): p. 485-92.

43. Berenson, G.S., et al., Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med, 1998. 338(23): p. 1650-6.

44. Ford, E.S., A.H. Mokdad, and U.A. Ajani, Trends in risk factors for cardiovascular disease among children and adolescents in the United States. Pediatrics, 2004. 114(6): p. 1534-44.

45. Brady, T.M.F., B.;Parekh, R. S.;Flynn, J. T., Racial Differences Among Children With Primary Hypertension. Pediatrics, 2010. 126(5): p. 931-937.

46. Berenson, G.S., et al., Risk factors in early life as predictors of adult heart disease: the Bogalusa Heart Study. Am J Med Sci, 1989. 298(3): p. 141-51.

47. Aglony, M., M. Acevedo, and G. Ambrosio, Hypertension in adolescents. Expert Rev Cardiovasc Ther, 2009. 7(12): p. 1595-603.

48. Hansen, M.L., P.W. Gunn, and D.C. Kaelber, Underdiagnosis of hypertension in children and adolescents. JAMA, 2007. 298(8): p. 874-9.

49. Beacher, D.R., et al., Recognition of elevated blood pressure in an outpatient pediatric tertiary care setting. J Pediatr, 2015. 166(5): p. 1233-1239 e1.

50. Whincup, P.H., D.G. Cook, and A.G. Shaper, Blood pressure measurement in children: the importance of cuff bladder size. J Hypertens, 1989. 7(10): p. 845-50.

51. Becton, L.J., et al., Blood pressure reclassification in adolescents based on repeat clinic blood pressure measurements. J Clin Hypertens (Greenwich), 2013. 15(10): p. 717-22.

52. Voors, A.W., L.S. Webber, and G.S. Berenson, Resting heart rate and pressure-rate product of children in a total biracial community: the Bogalusa Heart Study. Am J Epidemiol, 1982. 116(2): p. 276-86.

53. Gill, D.G., et al., Analysis of 100 children with severe and persistent hypertension. Arch Dis Child, 1976. 51(12): p. 951-6.

54. Tu, W., et al., Intensified effect of adiposity on blood pressure in overweight and obese children. Hypertension, 2011. 58(5): p. 818-24.

55. Lauer, R.M. and W.R. Clarke, Childhood risk factors for high adult blood pressure: the Muscatine Study. Pediatrics, 1989. 84(4): p. 633-41.

56. Still, J.L. and D. Cottom, Severe hypertension in childhood. Arch Dis Child, 1967. 42(221): p. 34-9.

57. Chu, P.Y., et al., Anti-hypertensive drugs in children and adolescents. World J Cardiol, 2014. 6(5): p. 234-44.

58. Spagnolo, A., et al., Focus on prevention, diagnosis and treatment of hypertension in children and adolescents. Ital J Pediatr, 2013. 39: p. 20.

59. Lande, M.B. and J.T. Flynn, Treatment of hypertension in children and adolescents. Pediatr Nephrol, 2009. 24(10): p. 1939-49.

60. Pescatello, L.S., et al., American College of Sports Medicine position stand. Exercise and hypertension. Med Sci Sports Exerc, 2004. 36(3): p. 533-53.

61. Hagberg, J.M., et al., Effect of exercise training on the blood pressure and hemodynamic features of hypertensive adolescents. Am J Cardiol, 1983. 52(7): p. 763-8.

62. Rocchini, A.P., et al., Blood pressure in obese adolescents: effect of weight loss. Pediatrics, 1988. 82(1): p. 16-23.

63. Anyaegbu, E.I.D., V. R., Hypertension in the teenager. Pediatric Clinics of North America, 2014. 61(1): p. 131-151.

64. Williams, R.R., et al., Are there interactions and relations between genetic and environmental factors predisposing to high blood pressure? Hypertension, 1991. 18(3 Suppl): p. I29-37.

65. Biron, P., J.G. Mongeau, and D. Bertrand, Familial aggregation of blood pressure in 558 adopted children. Can Med Assoc J, 1976. 115(8): p. 773-4.

66. Munoz, M., et al., Evaluating the contribution of genetics and familial shared environment to common disease using the UK Biobank. Nat Genet, 2016. 48(9): p. 980-3.

67. Burgos, M.S., et al., Relationship between anthropometric measures and cardiovascular risk factors in children and adolescents. Arq Bras Cardiol, 2013. 101(4): p. 288-96.

68. Zinner, S.H., et al., Significance of blood pressure in infancy. Familial aggregation and predictive effect on later blood pressure. Hypertension, 1985. 7(3 Pt 1): p. 411-6.

69. Bovet, P., et al., Prevalence of cardiovascular risk factors in a middle-income country and estimated cost of a treatment strategy. BMC Public Health, 2006. 6: p. 9.

70. Schack-Nielsen, L., C. Holst, and T.I. Sorensen, Blood pressure in relation to relative weight at birth through childhood and youth in obese and non-obese adult men. Int J Obes Relat Metab Disord, 2002. 26(12): p. 1539-46.

71. Adair, L.S., et al., Size at birth, weight gain in infancy and childhood, and adult blood pressure in 5 low- and middle-income-country cohorts: when does weight gain matter? Am J Clin Nutr, 2009. 89(5): p. 1383-92.

72. Woelk, G.B., Is low birth weight a risk factor for adult hypertension? A literature review with particular reference to Africa. South African Medical Journal. Suid-Afrikaanse Tydskrif Vir Geneeskunde, 1995. 85(12 Pt 2): p. 1348-9, 1352-3.

73. Barker, D.J., et al., Fetal and placental size and risk of hypertension in adult life. BMJ, 1990. 301(6746): p. 259-62.

74. Huxley, R.R., A.W. Shiell, and C.M. Law, The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. J Hypertens, 2000. 18(7): p. 815-31.

75. Law, C.M., et al., Maternal and fetal influences on blood pressure. Arch Dis Child, 1991. 66(11): p. 1291-5.

76. Sorof, J.M., et al., Overweight, ethnicity, and the prevalence of hypertension in school-aged children. Pediatrics, 2004. 113(3 Pt 1): p. 475-82.

77. Paciencia, I., et al., Association between sleep duration and blood pressure in adolescents. Hypertens Res, 2013. 36(8): p. 747-52.

78. Cournil, A., et al., Enhanced post-natal growth is associated with elevated blood pressure in young Senegalese adults. Int J Epidemiol, 2009. 38(5): p. 1401-10.

79. Belfort, M.B., et al., Size at birth, infant growth, and blood pressure at three years of age. J Pediatr, 2007. 151(6): p. 670-4.

80. Bowers, K., et al., Birth weight, postnatal weight change, and risk for high blood pressure among chinese children. Pediatrics, 2011. 127(5): p. e1272-9.

81. Steyn, K., et al., Cardiovascular disease risk factors in 5-year-old urban South African children--the Birth to Ten Study. S Afr Med J, 2000. 90(7): p. 719-26.

82. Willig, A.L., et al., Adjusting adiposity and body weight measurements for height alters the relationship with blood pressure in children. Am J Hypertens, 2010. 23(8): p. 904-10.

83. Guwatudde, D., et al., The Epidemiology of Hypertension in Uganda: Findings from the National Non-Communicable Diseases Risk Factor Survey. PLoS One, 2015. 10(9): p. e0138991.

84. Guwatudde, D., et al., The burden of hypertension in sub-Saharan Africa: a four-country cross sectional study. BMC Public Health, 2015. 15(1): p. 1211.

85. Leng, B., et al., Socioeconomic status and hypertension: a meta-analysis. J Hypertens, 2015. 33(2): p. 221-9.

86. Ansa, V.O., et al., Relationship between parental socio-economic status and casual blood pressure in coastal Nigerian adolescents. West Afr J Med, 2010. 29(3): p. 146-52.

87. Afrifa-Anane, E., et al., The association of physical activity, body mass index and the blood pressure levels among urban poor youth in Accra, Ghana. BMC Public Health, 2015. 15: p. 269.

88. Chen, J.L., et al., Risk factors for obesity and high blood pressure in Chinese American children: maternal acculturation and children's food choices. J Immigr Minor Health, 2011. 13(2): p. 268-75.

89. Brown, I.J., et al., Salt intakes around the world: implications for public health. Int J Epidemiol, 2009. 38(3): p. 791-813.

90. Okoh, B.A.N. and E.A.D. Alikor, Childhood hypertension and family history of hypertension in primary school children in Port Harcourt. Niger J Paed, 2013. 40(2): p. 184-188.

91. McCormick, M.C., The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med, 1985. 312(2): p. 82-90.

92. Lule, S.A., et al., Is birth weight associated with blood pressure among African children and adolescents? A systematic review. J Dev Orig Health Dis, 2018. 9(3): p. 270-280.

93. Monyeki, K., et al., Association between Blood Pressure and Birth Weight among Rural South African Children: Ellisras Longitudinal Study. Int J Environ Res Public Health, 2017. 14(9).

94. Hardy, R., et al., Birthweight and blood pressure in five European birth cohort studies: an investigation of confounding factors. Eur J Public Health, 2006. 16(1): p. 21-30.

95. Law, C.M., et al., Body size at birth and blood pressure among children in developing countries. Int J Epidemiol, 2000. 30(1): p. 52-7.

96. Falkner, B., S. Hulman, and H. Kushner, Effect of birth weight on blood pressure and body size in early adolescence. Hypertension, 2004. 43(2): p. 203-7.

97. Strufaldi, M.W., et al., Blood pressure levels in childhood: probing the relative importance of birth weight and current size. Eur J Pediatr, 2009. 168(5): p. 619-24.

98. Law, C.M. and A.W. Shiell, Is blood pressure inversely related to birth weight? The strength of evidence from a systematic review of the literature. J Hypertens, 1996. 14(8): p. 935-41.

99. Juonala, M., et al., Effect of birth weight on life-course blood pressure levels among children born premature: the Cardiovascular Risk in Young Finns Study. J Hypertens, 2015. 33(8): p. 1542-8.

100. Zhang, Y., et al., The associations of high birth weight with blood pressure and hypertension in later life: a systematic review and meta-analysis. Hypertens Res, 2013. 36(8): p. 725-35.

101. Launer, L.J., A. Hofman, and D.E. Grobbee, Relation between birth weight and blood pressure: longitudinal study of infants and children. BMJ, 1993. 307(6917): p. 1451-4.

102. Campbell, D.M., et al., Diet in pregnancy and the offspring's blood pressure 40 years later. Br J Obstet Gynaecol, 1996. 103(3): p. 273-80.

103. Macintyre, S., et al., Correlates of blood pressure in 15 year olds in the west of Scotland. J Epidemiol Community Health, 1991. 45(2): p. 143-7.

104. Hemachandra, A.H.K., M. A.;Furth, S. L., Racial disparities in the association between birth weight in the term infant and blood pressure at age 7 years: results from the collaborative perinatal project. Journal of the American Society of Nephrology, 2006. 17(9): p. 2576-81.

105. Rostand, S.G., S.P. Cliver, and R.L. Goldenberg, Racial disparities in the association of foetal growth retardation to childhood blood pressure. Nephrol Dial Transplant, 2005. 20(8): p. 1592-7.

106. Woelk, G., et al., Birthweight and blood pressure among children in Harare, Zimbabwe. Arch Dis Child Fetal Neonatal Ed, 1998. 79(2): p. F119-22.

107. Alves, J.G., J.N. Vilarim, and J.N. Figueiroa, Fetal influences on neonatal blood pressure. J Perinatol, 1999. 19(8 Pt 1): p. 593-5.

108. Cheung, Y.B., et al., Fetal growth and early postnatal growth are related to blood pressure in adults. Hypertension, 2000. 36(5): p. 795-800.

109. Whincup, P.H., et al., Size at birth and blood pressure at 3 years of age. The Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC). Am J Epidemiol, 1999. 149(8): p. 730-9.

110. Wen, X.Z.T., E. W.;Hogan, J. W.;Shenassa, E. D.;Buka, S. L., Association Between Placental Morphology and Childhood Systolic Blood Pressure. Hypertension, 2011. 57(1): p. 48-55.

111. Whincup, P.H., D.G. Cook, and O. Papacosta, Do maternal and intrauterine factors influence blood pressure in childhood? Arch Dis Child, 1992. 67(12): p. 1423-9.

112. Siewert-Delle, A. and S. Ljungman, The impact of birth weight and gestational age on blood pressure in adult life: a population-based study of 49-year-old men. Am J Hypertens, 1998. 11(8 Pt 1): p. 946-53.

113. Law, C.M., et al., Fetal, infant, and childhood growth and adult blood pressure: a longitudinal study from birth to 22 years of age. Circulation, 2002. 105(9): p. 1088-92.

114. Chiolero, A., et al., Birth weight, weight change, and blood pressure during childhood and adolescence: a school-based multiple cohort study. Journal of Hypertension, 2011. 29(10): p. 1871-1879.

115. Dasinger, J.H. and B.T. Alexander, Gender differences in developmental programming of cardiovascular diseases. Clin Sci (Lond), 2016. 130(5): p. 337-48.

116. de Almeida, F.A., et al., Assessment of social and economic influences on blood pressure of adolescents in public and private schools: an epidemiological study. J Bras Nefrol, 2011. 33(2): p. 142-9.

117. Beevers, D.G. and J.S. Prince, Some recent advances in non-communicable diseases in the Tropics. 1. Hypertension: an emerging problem in tropical countries. Trans R Soc Trop Med Hyg, 1991. 85(3): p. 324-6.

118. Etyang, A.O., Determining the Causal Role of Malaria in Elevating Blood Pressure and Pulse Wave Velocity in Kenyan Adolescents and Adults, in Department of Infectious Disease Epidemiology. 2017, London School of Hygiene & Tropical Medicine.

119. Bedu-Addo, G., et al., In utero exposure to malaria is associated with metabolic traits in adolescence: The Agogo 2000 birth cohort study. J Infect, 2017. 75(5): p. 455-463.

120. Nguyen, S., et al., Sugar-sweetened beverages, serum uric acid, and blood pressure in adolescents. J Pediatr, 2009. 154(6): p. 807-13.

121. Mellendick, K., et al., Diets Rich in Fruits and Vegetables Are Associated with Lower Cardiovascular Disease Risk in Adolescents. Nutrients, 2018. 10(2).

122. Yang, Q., et al., Sodium intake and blood pressure among US children and adolescents. Pediatrics, 2012. 130(4): p. 611-9.

123. Creating an enabling environment for population-based salt reduction strategies: report of a joint technical meeting held by WHO and the Food Standards Agency, United Kingdom, Geneva, World health Organization, 2010.

124. Sorof, J. and S. Daniels, Obesity hypertension in children: a problem of epidemic proportions. Hypertension, 2002. 40(4): p. 441-7.

125. Beratis, N.G., D. Panagoulias, and A. Varvarigou, Increased blood pressure in neonates and infants whose mothers smoked during pregnancy. J Pediatr, 1996. 128(6): p. 806-12.

126. Sanchez Bayle, M., et al., [A longitudinal study of physical exercise practice in children. The influence of age, gender and socioeconomic level. The Working Group on Cardiovascular Risk Factors in Childhood and Adolescence]. An Esp Pediatr, 1998. 48(1): p. 25-7.

127. Javaheri, S., et al., Sleep quality and elevated blood pressure in adolescents. Circulation, 2008. 118(10): p. 1034-40.

128. Wells, J.C., et al., Sleep patterns and television viewing in relation to obesity and blood pressure: evidence from an adolescent Brazilian birth cohort. Int J Obes (Lond), 2008. 32(7): p. 1042-9.

129. Robinson, R.F., et al., Significance of heritability in primary and secondary pediatric hypertension. Am J Hypertens, 2005. 18(7): p. 917-21.

130. Vattikuti, S., J. Guo, and C.C. Chow, Heritability and genetic correlations explained by common SNPs for metabolic syndrome traits. PLoS Genet, 2012. 8(3): p. e1002637.

131. Zhu, X., et al., Admixture mapping for hypertension loci with genome-scan markers. Nat Genet, 2005. 37(2): p. 177-81.

132. Cutler, J.A., et al., Trends in hypertension prevalence, awareness, treatment, and control rates in United States adults between 1988-1994 and 1999-2004. Hypertension, 2008. 52(5): p. 818-27.

133. Harding, S., et al., Emergence of ethnic differences in blood pressure in adolescence: the determinants of adolescent social well-being and health study. Hypertension, 2010. 55(4): p. 1063-9.

134. Warren, H.R., et al., Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. Nat Genet, 2017. 49(3): p. 403-415.

135. Xi, B., et al., Association of common variants in/near six genes (ATP2B1, CSK, MTHFR, CYP17A1, STK39 and FGF5) with blood pressure/hypertension risk in Chinese children. J Hum Hypertens, 2014. 28(1): p. 32-6.

136. Levy, D., et al., Genome-wide association study of blood pressure and hypertension. Nat Genet, 2009. 41(6): p. 677-87.

137. Qian, X., et al., A meta-analysis of association between C677T polymorphism in the methylenetetrahydrofolate reductase gene and hypertension. Eur J Hum Genet, 2007. 15(12): p. 1239-45.

138. Cho, Y.S., et al., A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. Nat Genet, 2009. 41(5): p. 527-34.

139. Fox, E.R., et al., Association of genetic variation with systolic and diastolic blood pressure among African Americans: the Candidate Gene Association Resource study. Hum Mol Genet, 2011. 20(11): p. 2273-84.

140. Chang, Y.P., et al., Multiple genes for essential-hypertension susceptibility on chromosome 1q. Am J Hum Genet, 2007. 80(2): p. 253-64.

141. Ferrandi, M., et al., Renal Na,K-ATPase in genetic hypertension. Hypertension, 1996. 28(6): p. 1018-25.

142. Yang, B., et al., Associations of MTHFR gene polymorphisms with hypertension and hypertension in pregnancy: a meta-analysis from 114 studies with 15411 cases and 21970 controls. PLoS One, 2014. 9(2): p. e87497.

143. Delpire, E. and K.B. Gagnon, SPAK and OSR1: STE20 kinases involved in the regulation of ion homoeostasis and volume control in mammalian cells. Biochem J, 2008. 409(2): p. 321-31.

144. Kidambi, S., et al., Non-replication study of a genome-wide association study for hypertension and blood pressure in African Americans. BMC Med Genet, 2012. 13: p. 27.

145. Franceschini, N., et al., Genome-wide association analysis of blood-pressure traits in African-ancestry individuals reveals common associated genes in African and non-African populations. Am J Hum Genet, 2013. 93(3): p. 545-54.

146. Adeyemo, A., et al., A genome-wide association study of hypertension and blood pressure in African Americans. PLoS Genet, 2009. 5(7): p. e1000564.

147. Salfati, E., et al., Direct Estimates of the Genomic Contributions to Blood Pressure Heritability within a Population-Based Cohort (ARIC). PLoS One, 2015. 10(7): p. e0133031.

148. Ehret, G.B. and M.J. Caulfield, Genes for blood pressure: an opportunity to understand hypertension. Eur Heart J, 2013. 34(13): p. 951-61.

149. Manolio, T.A., et al., Finding the missing heritability of complex diseases. Nature, 2009. 461(7265): p. 747-53.

150. Maher, B., Personal genomes: The case of the missing heritability. Nature, 2008. **456**(7218): p. 18-21.

151. Barker, D.J. and C. Osmond, Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet, 1986. **1**(8489): p. 1077-81.

152. Law, C.M., et al., Initiation of hypertension in utero and its amplification throughout life. BMJ, 1993. **306**(6869): p. 24-7.

153. Blake, K.V., et al., Adjustment for current weight and the relationship between birth weight and blood pressure in childhood. J Hypertens, 2000. **18**(8): p. 1007-12.

154. Pharoah, P.O., C.J. Stevenson, and C.R. West, Association of blood pressure in adolescence with birthweight. Arch Dis Child Fetal Neonatal Ed, 1998. **79**(2): p. F114-8.

155. Ludwig, D.S. and J. Currie, The association between pregnancy weight gain and birthweight: a within-family comparison. Lancet, 2010. **376**(9745): p. 984-90.

156. Han, Z., et al., Maternal underweight and the risk of preterm birth and low birth weight: a systematic review and meta-analyses. Int J Epidemiol, 2011. **40**(1): p. 65-101.

157. Roseboom, T., S. de Rooij, and R. Painter, The Dutch famine and its long-term consequences for adult health. Early Hum Dev, 2006. **82**(8): p. 485-91.

158. Roseboom, T.J., et al., Maternal nutrition during gestation and blood pressure in later life. J Hypertens, 2001. **19**(1): p. 29-34.

159. Nuyt, A.M., Mechanisms underlying developmental programming of elevated blood pressure and vascular dysfunction: evidence from human studies and experimental animal models. Clin Sci (Lond), 2008. **114**(1): p. 1-17.

160. Tobi, E.W., et al., DNA methylation signatures link prenatal famine exposure to growth and metabolism. Nat Commun, 2014. **5**: p. 5592.

161. Vagero, D. and D. Leon, Ischaemic heart disease and low birth weight: a test of the fetal-origins hypothesis from the Swedish Twin Registry. Lancet, 1994. **343**(8892): p. 260-3.

162. Fall, C.H., Fetal programming and the risk of noncommunicable disease. Indian J Pediatr, 2013. **80 Suppl 1**: p. S13-20.

163. Alexander, B.T., Placental insufficiency leads to development of hypertension in growth-restricted offspring. Hypertension, 2003. **41**(3): p. 457-62.

164. Langley-Evans, S.C., Fetal programming of cardiovascular function through exposure to maternal undernutrition. Proceedings of the Nutrition Society, 2007. **60**(04): p. 505-513.

165. Tang, L., et al., Gender differences in the effects of antenatal betamethasone exposure on renal function in adult sheep. Am J Physiol Regul Integr Comp Physiol, 2009. **296**(2): p. R309-17.

166. Celsi, G., et al., Prenatal dexamethasone causes oligonephronia, sodium retention, and higher blood pressure in the offspring. Pediatr Res, 1998. **44**(3): p. 317-22.

167. Gardner, D.S., A.A. Jackson, and S.C. Langley-Evans, The effect of prenatal diet and glucocorticoids on growth and systolic blood pressure in the rat. Proc Nutr Soc, 1998. **57**(2): p. 235-40.

168. Vickers, M.H., S.O. Krechowec, and B.H. Breier, Is later obesity programmed in utero? Curr Drug Targets, 2007. **8**(8): p. 923-34.

169. Harding, J.E., The nutritional basis of the fetal origins of adult disease. Int J Epidemiol, 2001. **30**(1): p. 15-23.

170. Tomat, A.L. and F.J. Salazar, Mechanisms involved in developmental programming of hypertension and renal diseases. Gender differences. Horm Mol Biol Clin Investig, 2014. **18**(2): p. 63-77.

171. Harding, J., et al., Intrauterine feeding of the growth retarded fetus: can we help? Early Hum Dev, 1992. **29**(1-3): p. 193-7.

172. Hoet, J.J. and M.A. Hanson, Intrauterine nutrition: its importance during critical periods for cardiovascular and endocrine development. J Physiol, 1999. **514**(6): p. 617-27.

173. Langley-Evans, S.C., A.J. Langley-Evans, and M.C. Marchand, Nutritional programming of blood pressure and renal morphology. Arch Physiol Biochem, 2003. **111**(1): p. 8-16.

174. Hales, C.N. and D.J. Barker, The thrifty phenotype hypothesis. Br Med Bull, 2001. **60**: p. 5-20.

175. Rudolph, A.M., The fetal circulation and its response to stress. J Dev Physiol, 1984. **6**(1): p. 11-9.

176. Woods, L.L., D.A. Weeks, and R. Rasch, Programming of adult blood pressure by maternal protein restriction: role of nephrogenesis. Kidney Int, 2004. **65**(4): p. 1339-48.

177. Welham, S.J., A. Wade, and A.S. Woolf, Protein restriction in pregnancy is associated with increased apoptosis of mesenchymal cells at the start of rat metanephrogenesis. Kidney Int, 2002. **61**(4): p. 1231-42.

178. Pham, T.D., et al., Uteroplacental insufficiency increases apoptosis and alters p53 gene methylation in the full-term IUGR rat kidney. Am J Physiol Regul Integr Comp Physiol, 2003. **285**(5): p. R962-70.

179. Gilbert, J.S., et al., Maternal nutrient restriction in sheep: hypertension and decreased nephron number in offspring at 9 months of age. J Physiol, 2005. **565**(Pt 1): p. 137-47.

180. Langley-Evans, S.C., S.J. Welham, and A.A. Jackson, Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. Life Sci, 1999. **64**(11): p. 965-74.

181. Vehaskari, V.M., D.H. Aviles, and J. Manning, Prenatal programming of adult hypertension in the rat. Kidney Int, 2001. **59**(1): p. 238-45.

182. Bassan, H., et al., Experimental intrauterine growth retardation alters renal development. Pediatr Nephrol, 2000. **15**(3-4): p. 192-5.

183. Merlet-Benichou, C., et al., Intrauterine growth retardation leads to a permanent nephron deficit in the rat. Pediatr Nephrol, 1994. **8**(2): p. 175-80.

184. Hinchliffe, S.A., et al., The effect of intrauterine growth retardation on the development of renal nephrons. Br J Obstet Gynaecol, 1992. **99**(4): p. 296-301.

185. Ojeda, N.B., D. Grigore, and B.T. Alexander, Developmental programming of hypertension: insight from animal models of nutritional manipulation. Hypertension, 2008. **52**(1): p. 44-50.

186. Brennan, K.A., D.M. Olson, and M.E. Symonds, Maternal nutrient restriction alters renal development and blood pressure regulation of the offspring. Proc Nutr Soc, 2006. **65**(1): p. 116-24.

187. Gilbert, J.S. and M.J. Nijland, Sex differences in the developmental origins of hypertension and cardiorenal disease. Am J Physiol Regul Integr Comp Physiol, 2008. **295**(6): p. R1941-52.

188. Grigore, D., N.B. Ojeda, and B.T. Alexander, Sex differences in the fetal programming of hypertension. Gend Med, 2008. **5 Suppl A**: p. S121-32.

189. Salazar, F., et al., Age- and sodium-sensitive hypertension and sex-dependent renal changes in rats with a reduced nephron number. Hypertension, 2008. **51**(4): p. 1184-9.

190. Seckl, J.R., Glucocorticoids, feto-placental 11 beta-hydroxysteroid dehydrogenase type 2, and the early life origins of adult disease. Steroids, 1997. **62**(1): p. 89-94.

191. Clark, P.M., et al., Size at birth and adrenocortical function in childhood. Clin Endocrinol (Oxf), 1996. **45**(6): p. 721-6.

192. Warner, M.J. and S.E. Ozanne, Mechanisms involved in the developmental programming of adulthood disease. Biochem J, 2010. **427**(3): p. 333-47.

193. Isgaard, J., Cardiovascular disease and risk factors: the role of growth hormone. Horm Res, 2004. **62 Suppl 4**: p. 31-8.

194. Gambling, L., et al., Iron deficiency during pregnancy affects postnatal blood pressure in the rat. J Physiol, 2003. **552**(Pt 2): p. 603-10.

195. Lewis, R.M., et al., Effects of maternal iron restriction in the rat on blood pressure, glucose tolerance, and serum lipids in the 3-month-old offspring. Metabolism, 2001. **50**(5): p. 562-7.

196. Swanson, J.M., et al., Developmental origins of health and disease: environmental exposures. Semin Reprod Med, 2009. **27**(5): p. 391-402.

197. Webster, A.L., M.S. Yan, and P.A. Marsden, Epigenetics and cardiovascular disease. Can J Cardiol, 2013. **29**(1): p. 46-57.

198. Sinclair, K.D., et al., DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. Proc Natl Acad Sci U S A, 2007. **104**(49): p. 19351-6.

199. Weaver, I.C., et al., Epigenetic programming by maternal behavior. Nat Neurosci, 2004. **7**(8): p. 847-54.

200. Tain, Y.L. and J.A. Joles, Reprogramming: A Preventive Strategy in Hypertension Focusing on the Kidney. Int J Mol Sci, 2015. **17**(1).

201. Harrap, S.B., Where are all the blood-pressure genes? Lancet, 2003. **361**(9375): p. 2149-51.

202. Pande, J., et al., Aortic smooth muscle and endothelial plasma membrane Ca2+ pump isoforms are inhibited differently by the extracellular inhibitor caloxin 1b1. Am J Physiol Cell Physiol, 2006. **290**(5): p. C1341-9.

203. Monteith, G.R., et al., Elevated plasma membrane and sarcoplasmic reticulum Ca2+ pump mRNA levels in cultured aortic smooth muscle cells from spontaneously hypertensive rats. Biochem Biophys Res Commun, 1997. **230**(2): p. 344-6.

204. Akombi, B.J., et al., Child malnutrition in sub-Saharan Africa: A meta-analysis of demographic and health surveys (2006-2016). PLoS One, 2017. **12**(5): p. e0177338.

205. Vorster, H.H. and A. Kruger, Poverty, malnutrition, underdevelopment and cardiovascular disease: a South African perspective. Cardiovasc J Afr, 2007. **18**(5): p. 321-4.

206. Benzekri, N.A., et al., High Prevalence of Severe Food Insecurity and Malnutrition among HIV-Infected Adults in Senegal, West Africa. PLoS One, 2015. **10**(11): p. e0141819.

207. Delnord, M., B. Blondel, and J. Zeitlin, What contributes to disparities in the preterm birth rate in European countries? Curr Opin Obstet Gynecol, 2015. **27**(2): p. 133-42.

208. Ay, L., et al., Maternal anthropometrics are associated with fetal size in different periods of pregnancy and at birth. The Generation R Study. BJOG, 2009. **116**(7): p. 953-63.

209. Garner, P., M.S. Kramer, and I. Chalmers, Might efforts to increase birthweight in undernourished women do more harm than good? Lancet, 1992. **340**(8826): p. 1021-3.

210. Muchemi, O.M., E. Echoka, and A. Makokha, Factors associated with low birth weight among neonates born at Olkalou District Hospital, Central Region, Kenya. Pan Afr Med J, 2015. **20**: p. 108.

211. Tishkoff, S.A., et al., The genetic structure and history of Africans and African Americans. Science, 2009. **324**(5930): p. 1035-44.

212. Luoni, G., et al., Population-specific patterns of linkage disequilibrium in the human 5q31 region. Genes Immun, 2005. **6**(8): p. 723-7.

Chapter 2: Thesis aim, objectives, design and structure, candidate's contribution and list of associated publications

2.1 Introduction

This chapter presents the thesis aim, objectives, design and structure and the candidate's role and contribution to this thesis.

2.2 Thesis aim

The aim of this thesis was to investigate the influence of birth weight, postnatal growth, pre-natal, peri-natal and childhood exposures and of genetic polymorphisms on BP among young Africans.

2.3 Thesis objectives

- **Thesis objective 1:** Review evidence on the relationship between birth weight and BP among African children and adolescents
- **Thesis objective 2:** Investigate the relationship between birth weight and BP among Ugandan adolescents
- **Thesis objective 3:** Investigate the relationship between postnatal growth and BP among Ugandan adolescents
- **Thesis objective 4:** Identify other factors associated with BP among Ugandan adolescents
- **Thesis objective 5**: Investigate genetic polymorphisms associated with BP among Ugandan adolescents

Thesis objective 1 was assessed through a systematic review, while thesis objectives 2-5 were assessed through analysis of longitudinal data collected prospectively in the EMaBS birth cohort of 2,345 live born offspring in Wakiso district, Uganda.

2.4 Thesis design and structure

2.4.1 Thesis design

This PhD was designed to use both a systematic review and an observational study to describe early-life, life-course and genetic factors associated with BP in young Africans. The systematic review assesses published literature on the relationship between birth weight and BP among African children and adolescents (aged 0 to 18 years). The

observational study uses prospectively collected longitudinal data from the EMaBS birth cohort (http://emabs.lshtm.ac.uk/) to examine the impact of early-life (fetal and postnatal period) factors, life-course factors and genetic polymorphisms on BP in adolescents.

2.4.2 Thesis structure

This thesis comprises of eight chapters and is written following the 'research paper' style format, with five research papers associated with this PhD project. Each research paper was prepared as a stand-alone manuscript and thus there is unavoidably repetition of information (such as setting, definitions) among research papers. The research papers are presented in an order that makes this thesis cohesive, rather than in the temporal order of publication.

- Chapter 1: The background chapter reviews the literature on the epidemiology of BP in children and adolescents, presents the hypothesis, describes current understanding of the mechanisms for the developmental programming of later BP and summarises the justification for this PhD.
- Chapter 2: Summarises the thesis aims, objectives, design and structure, and the candidate's role and contributions
- Chapter 3: The systematic review describes the methods and the findings for thesis objective 1
- Chapter 4: The methods defines and classifies the terms used throughout the thesis and describes the methods used to answer thesis objectives 2, 3, 4 and 5
- Chapter 5: Addresses thesis objective 2 "investigate the relationship between birth weight and BP among Ugandan adolescents" and thesis objective 3 "investigate the relationship between postnatal growth and BP among Ugandan adolescents"
- Chapter 6: Addresses thesis objective 4 "identify other factors associated with BP among Ugandan adolescents"
- Chapter 7: Addresses thesis objective 5 "investigate genetic polymorphisms associated with BP among Ugandan adolescents"
- Chapter 8: The discussion summarises the PhD findings in relation to the existing literature, implications of the findings, strengths, limitations, proposed future work and conclusions
- Appendices contain important documents relevant to this thesis including ethical approval and notification, information, consent and assent forms and an extra manuscript (not part of the thesis, hence not included in the main body) produced

during the course of the PhD in collaboration with Jonathan Nsamba, a master's student at the LSHTM, which examines association between early-life factors and body composition in early adolescence

2.5 Candidate's contribution

Since joining the MRC/UVRI & LSHTM in 2006, I have had the opportunity to serve in various roles and contribute substantially to the clinical follow-up of the EMaBS birth cohort.

2.5.1 Entebbe Mother and Baby Study birth cohort

I worked as a Medical Officer between 2006 and 2011 and my roles included provision of medical care (taking medical history, conducting medical examinations and providing necessary treatment) to study participants; obtaining informed consent; interviewing study participants and completing study questionnaires; reporting adverse and serious adverse events to regulatory authorities; data collection, management and cleaning.

From 2011 to 2015, I was a Project Leader. My responsibilities were to prepare, compile and submit study progress reports to management and regulatory authorities; procure study supplies, equipment and consumables; train study staff and attached students; develop statistical analysis plans; conduct and interpret data analysis; and disseminate study results.

Between 2013 and 2015, I was involved with the initiation, design and conduct of the BP study, nested within the EMaBS. I was responsible for developing study documents (including protocol, questionnaires, standard operating procedures (SOP), study information sheets, consent and assent forms); obtaining ethical approvals (including subsequent renewals and amendments) from the regulatory authorities; reporting study progress to local regulatory authorities; staff training and supervision; data collection, management and cleaning; ensuring that the protocol was adhered to; and carrying out administrative duties related to the study.

2.5.2 PhD work

PhD registration was in January 2016

2.5.2.1 Role in the systematic review included:

1. Developed the systematic review protocol with guidance from my supervisors

2. Performed database search and retrieved publications for inclusion in the review process
3. Together with my supervisor we independently screened and assessed titles and abstracts for inclusion in the full-text review, assessed full-text articles for inclusion in the review, extracted data from selected manuscripts and discussed inconsistencies with consensus reached at each stage in the selection process.
4. Drafted and revised the systematic review manuscript following comments from the co-authors
5. Submitted the final version of the manuscript for publication and responded to reviewers' comments in consultation with the co-authors

2.5.2.2 Role in the longitudinal observational study included:

1. Conducted extensive data management and cleaning, which included generating and resolving data queries and updating databases and archived case report forms (CRF) copies, merging datasets and generating a single analysis dataset
2. Developed data analysis plan and conducted data analysis for thesis objectives 2, 3, 4, and 5
3. Drafted manuscripts arising from this work, incorporated feedback from my supervisors and co-authors
4. Submitted manuscripts for publication and responded to reviewers' comments
5. Presented study findings at various conferences and gatherings
6. Drafted the thesis report, revised, incorporated and responded to feedback from my supervisors

2.6 Associated publications

1. **Research paper 1**: Is birth weight associated with blood pressure among African children and adolescents? A systematic review, Journal of Developmental Origins of Health and Disease, 2018. 9(3): p. 270-280
2. **Research paper 2**: Are birth weight and postnatal weight gain in childhood associated with blood pressure in early adolescence? Results from a Ugandan birth cohort, International Journal of Epidemiology, 2018 (Epub ahead of print)
3. **Research paper 3**: Blood pressure risk factors in early adolescents, results from a Ugandan birth cohort, Journal of Human Hypertension (under review)

4. **Research paper 4**: A genome-wide association and replication study of blood pressure in Ugandan adolescents, draft in preparation for submission to Clinical Genetics
5. **Research paper 5**: Effect of birth weight, exclusive breastfeeding and growth rate in childhood on fat mass and fat-free mass indices in early adolescence: Results from a birth cohort, Current Developments in Nutrition (under review)

The systematic review protocol and research paper 1 presented in chapter 3 were written in accordance with guidelines from the Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) guidelines [1]. Research papers 2, 3, 4 and 5 from the observational study have been written following guidelines from the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [2].

2.7 Associated conferences and workshop presentations

1. Health of adolescents and young people in Africa, Mwanza Intervention Trials Unit (MITU) Scientific Symposium, 27th-29th November 2018, won one of the three best poster presentation prizes.
2. International Society of Hypertension Conference on the 20th-23rd September 2018, Beijing China (Poster Abstract ID: A5593)
3. The Uganda Virus Research Institute (UVRI) monthly seminar, Non-communicable Diseases theme day 27th April 2018 (Oral presentation)
4. The 5th Symposium of Makerere-Columbia (MUCU) on "Transition in Care: Children to adolescents to Young Adults" by the Society of Adolescent Health in Uganda 11th-12th April 2018 (Oral presentation)
5. London School of Hygiene Tropical Medicine poster day on the 15th March 2018
6. The 1st World Congress on Hypertension in Children and Adolescents, 9th-11th February 2018, Valencia, Spain (E-poster)
7. The Developmental Origin of Health and Disease, 15th-18th October 2017, Rotterdam, Netherlands (Poster number: PO1.02.04)

2.8 References

1. Moher, D., et al., Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev, 2015. **4**: p. 1.
2. Vandenbroucke, J.P., et al., Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. PLoS Med, 2007. **4**(10): p. e297.

Chapter 3: Systematic review

3.1 Introduction

The systematic review was used to answer thesis objective 1: "review evidence on the relationship between birth weight and blood pressure among African children and adolescents". This chapter describes the methods used to conduct the systematic review and presents findings of the systematic review. The systematic review protocol was developed in consultation with my supervisors prior to starting the review process. Findings from the systematic review are summarised in research paper 1 "Is birth weight associated with blood pressure among African children and adolescents? A systematic review", as published in the Journal of Developmental Origins of Health and Disease [1].

3.2 Methods: Protocol for a systematic review of the relationship between birth weight and blood pressure among African children and adolescents

Abstract

Introduction

An inverse relationship between birth weight and blood pressure (BP) has been reported by various studies in high-income countries. Despite the high prevalence and the diverse causes of low birth weight on the African continent, little has been reported on the relationship between birth weight and BP in young Africans. Understanding this relationship among young Africans has huge potential to inform policy to mitigate the escalating burden of high BP on the continent.

Aim: Review literature on the relationship between birth weight and BP among African children and adolescents.

Data source and extraction: The Medline, EMBASE, Global Health and Web of Science databases will be searched for potential publications. References for relevant articles will be scanned for additional potential articles. Results will be reported following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines.

Ethics: The review will be based on published studies; thus, no ethical approval is required. The study will provide evidence on the relationship between birth weight and BP, identify knowledge gaps and guide future research interests.

Dissemination

Findings will be summarised and published in a peer-reviewed journal.

Funding and role of the sponsor or funder

This work is partly supported by the Commonwealth Scholarship Commission (S.L., PhD funding at the LSHTM); the Wellcome Trust (A.E., grant number 095778, L.S., grant number 098504/Z/12/Z); and the UK Medical Research Council (E.W., grant number MR/K012126/1).

Neither the sponsor nor the funder(s) has participated in the protocol design.

Introduction

Hypertension, once uncommon on the African continent [2, 3], is a major risk factor for cardiovascular diseases (CVDs). Most people affected by hypertension are unaware of it, and among those that are aware, many are not on treatment [4-7]. Among African children and adolescents, high blood pressure (BP) prevalence has been reported at 10% in Seychelles[8], 17% in Uganda [9] and 21% in South Africa[10]. Nearly 75% of hypertension in children and adolescents is undiagnosed [11]. Hypertension, which contributes to CVDs mortality and morbidity in adulthood [12-16], has its origins in childhood [17-19]. Children with high BP tend to become hypertensive adults [20].

Extensive literature from high-income countries (HICs) suggests an inverse relationship between birth weight and BP later in life [21-25]. Previous systematic reviews on the relationship between birth weight and BP in children or adolescents have included mainly studies from HICs. On average, systolic BP increases by 2-4 mmHg for a one kg drop in birth weight [26, 27], supporting the fetal origin hypothesis proposed by David Barker [28]. Barker hypothesised that intra-uterine nutrition (often measured as birth weight) is vital in programming CVDs risk factors including BP. However, not all studies have reported an inverse relationship between birth weight and BP. Birth weight was positively associated with BP among neonates from Ireland [29], no association was seen among adolescents in the USA [30], and the relationship differed by gender among 15-year-olds from Scotland, with an inverse association observed for males but a positive association for females [31].

Overall, men have higher BP than age-matched women before menopause [32]. However, few epidemiological studies have investigated the effects of gender on developmental origin of BP despite strong evidence from numerous experimental studies that male

offspring exhibit increased BP in young adulthood while female offspring remain normotensive [32].

The birth weight-BP relationship remains unclear in Africa, where low birth weight is common [33, 34]. One may propose that among Africans, the birth weight-BP relationship might be different to that which is reported in developed countries. The causes and prevention strategies for low birth weight differ between rural, tropical Africa and developed or sub-tropical settings: for example, malaria (an important cause of low birth weight) is restricted to the tropics [35] and prophylactic antimalarial drugs in pregnancy reduce the risk of low birth weight [36].

It is with this background that we present a protocol for a systematic review to describe the relationship between birth weight and BP among young Africans. The review intends to discuss literature on the relationship between birth weight and BP among African children and adolescents, identify knowledge gaps and guide future research interests. The review may provide supporting evidence for future hypertension prevention strategies for Africa.

Objectives

The review aims to use published literature to examine the relationship between birth weight and BP among African children and adolescents. We aim to:

1) Outline the characteristics and findings of the studies
2) Describe the current evidence, gaps in evidence and the implications for future research and policies

Search strategy and data extraction

The protocol and the review will follow guidelines from the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) checklist and statement [37, 38]. We will perform the literature search in Medline, EMBASE and Global Health and Web of Science to the current date. The search will use key search terms to retrieve potentially relevant studies. The key search terms are (hypertension OR blood pressure) AND (birth weight) AND (paediatric OR child OR young people OR youth OR juvenile OR adolescent OR youngster OR pubescent OR teenage OR newborn OR minor OR infant) AND (Africa OR individual names of countries in Africa). Articles will be exported into EndNote, de-duplicated and then exported into an excel spreadsheet to permit the selection of articles and data extraction.

Two independent authors will assess titles and abstracts for inclusion in the full-text review and also independently assess full-text articles for inclusion in the data synthesis. Inconsistencies will be discussed, and consensus reached at each stage of the selection process. Reference lists will also be scanned for additional studies. Eligible papers will be accessed through PubMed, HINARI, LSHTM library or the journal's website. Information will be extracted independently by two authors using standardized data extraction sheets.

The review will include all primary studies on the relationship between birth weight and BP among young Africans (0 to 18 years), residing on the African continent. Studies that include both adults and children or adolescents will be included in the review if they clearly state the relationship between birth weight and BP in children and, or adolescents (ages 0 to 18 years), i.e. separately from results in adults.

Studies will be excluded if conducted among African populations residing outside the African continent, if studying non-systemic hypertension (such as pulmonary hypertension, intracranial hypertension), if not accessible within three months of sending requests to the authors and if including a wider age range but where it is not possible to disaggregate childhood or adolescence data.

No restriction on language or publication dates will be applied. Multiple articles from the same study will be reported as one study. Key information including study characteristics, population, design, authors, study area (region of Africa and country), sample size, BP measurement protocol, participants' year(s) of birth, sex, age, mean BP (systolic and or diastolic), mean birth weight and information on the direction and strength of evidence of association between birth weight and BP, and on potential confounders and effect modifiers will be gathered in tables.

No meta-analysis is planned because it is anticipated that there will be a substantial amount of heterogeneity in study populations, designs and methodologies. The study selection process will be summarised using a flow diagram. Reasons for exclusion will be described. Tables accompanied by narratives will be presented.

Ethics and dissemination

The review will be based on published data, therefore ethical approval is not required and will not be obtained. This systematic review will serve as a guide for future BP aetiological, prevention and control studies among young Africans residing on the continent. Results will be published in a peer-reviewed journal and in a PhD thesis.

Contributors

SAL conceived, designed and drafted the protocol, with input and reviews from AME, LS and ELW.

3.3 References

1. Lule, S.A., et al., Is birth weight associated with blood pressure among African children and adolescents? A systematic review. J Dev Orig Health Dis, 2018. **9**(3): p. 270-280.
2. Essouma, M., et al., Hypertension prevalence, incidence and risk factors among children and adolescents in Africa: a systematic review and meta-analysis protocol. BMJ Open, 2015. **5**(9): p. e008472.
3. Donnison, C.P., Pressure in the African Native: its bearing upon the aetiology of hyperpiesia and arteriosclerosis. The Lancet, 1929. **213**(5497): p. 6-7.
4. Ataklte, F., et al., Burden of undiagnosed hypertension in sub-saharan Africa: a systematic review and meta-analysis. Hypertension, 2015. **65**(2): p. 291-8.
5. Guwatudde, D., et al., The burden of hypertension in sub-Saharan Africa: a four-country cross sectional study. BMC Public Health, 2015. **15**(1): p. 1211.
6. Kotwani, P., et al., Epidemiology and awareness of hypertension in a rural Ugandan community: a cross-sectional study. BMC Public Health, 2013. **13**: p. 1151.
7. Addo, J., L. Smeeth, and D.A. Leon, Hypertension in sub-saharan Africa: a systematic review. Hypertension, 2007. **50**(6): p. 1012-8.
8. Chiolero, A., et al., Prevalence of elevated blood pressure and association with overweight in children of a rapidly developing country. J Hum Hypertens, 2007. **21**(2): p. 120-7.
9. Kidy, F., et al., Blood pressure in primary school children in Uganda: a cross-sectional survey. BMC Public Health, 2014. **14**(1): p. 1223.
10. Nkeh-Chungag, B.N., et al., Prevalence of hypertension and pre-hypertension in 13-17 year old adolescents living in Mthatha - South Africa: a cross-sectional study. Cent Eur J Public Health, 2015. **23**(1): p. 59-64.
11. Aglony, M., M. Acevedo, and G. Ambrosio, Hypertension in adolescents. Expert Rev Cardiovasc Ther, 2009. **7**(12): p. 1595-603.
12. Pastor-Barriuso, R., et al., Systolic blood pressure, diastolic blood pressure, and pulse pressure: an evaluation of their joint effect on mortality. Ann Intern Med, 2003. **139**(9): p. 731-9.

13. Antikainen, R.L., et al., Excess mortality associated with increased pulse pressure among middle-aged men and women is explained by high systolic blood pressure. J Hypertens, 2000. **18**(4): p. 417-23.

14. Almgren, T., et al., Stroke and coronary heart disease in treated hypertension -- a prospective cohort study over three decades. J Intern Med, 2005. **257**(6): p. 496-502.

15. Antikainen, R., P. Jousilahti, and J. Tuomilehto, Systolic blood pressure, isolated systolic hypertension and risk of coronary heart disease, strokes, cardiovascular disease and all-cause mortality in the middle-aged population. J Hypertens, 1998. **16**(5): p. 577-83.

16. Falkner, B., Hypertension in children and adolescents: epidemiology and natural history. Pediatr Nephrol, 2010. **25**(7): p. 1219-24.

17. Berenson, G.S., et al., Association between Multiple Cardiovascular Risk Factors and Atherosclerosis in Children and Young Adults. N Engl J Med, 1998. **338**(23): p. 1650-1656.

18. Lauer, R.M. and W.R. Clarke, Childhood risk factors for high adult blood pressure: the Muscatine Study. Pediatrics, 1989. **84**(4): p. 633-41.

19. McNiece, K.L., et al., Prevalence of hypertension and pre-hypertension among adolescents. J Pediatr, 2007. **150**(6): p. 640-4, 644 e1.

20. Flynn, J.T., Pediatric hypertension update. Curr Opin Nephrol Hypertens, 2010. **19**(3): p. 292-7.

21. Schack-Nielsen, L., C. Holst, and T.I. Sorensen, Blood pressure in relation to relative weight at birth through childhood and youth in obese and non-obese adult men. Int J Obes Relat Metab Disord, 2002. **26**(12): p. 1539-46.

22. Adair, L.S., et al., Size at birth, weight gain in infancy and childhood, and adult blood pressure in 5 low- and middle-income-country cohorts: when does weight gain matter? Am J Clin Nutr, 2009. **89**(5): p. 1383-92.

23. Law, C.M., et al., Initiation of hypertension in utero and its amplification throughout life. BMJ, 1993. **306**(6869): p. 24-7.

24. Blake, K.V., et al., Adjustment for current weight and the relationship between birth weight and blood pressure in childhood. J Hypertens, 2000. **18**(8): p. 1007-12.

25. Pharoah, P.O., C.J. Stevenson, and C.R. West, Association of blood pressure in adolescence with birthweight. Arch Dis Child Fetal Neonatal Ed, 1998. **79**(2): p. F114-8.

26. Law, C.M. and A.W. Shiell, Is blood pressure inversely related to birth weight? The strength of evidence from a systematic review of the literature. J Hypertens, 1996. **14**(8): p. 935-41.

27. Huxley, R.R., A.W. Shiell, and C.M. Law, The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. J Hypertens, 2000. **18**(7): p. 815-31.

28. Barker, D.J. and C. Osmond, Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet, 1986. **1**(8489): p. 1077-81.

29. O'Sullivan, M.J., P.J. Kearney, and M.J. Crowley, The influence of some perinatal variables on neonatal blood pressure. Acta Paediatr, 1996. **85**(7): p. 849-53.

30. Falkner, B., S. Hulman, and H. Kushner, Effect of birth weight on blood pressure and body size in early adolescence. Hypertension, 2004. **43**(2): p. 203-7.

31. Macintyre, S., et al., Correlates of blood pressure in 15 year olds in the west of Scotland. J Epidemiol Community Health, 1991. **45**(2): p. 143-7.

32. Dasinger, J.H. and B.T. Alexander, Gender differences in developmental programming of cardiovascular diseases. Clin Sci (Lond), 2016. **130**(5): p. 337-48.

33. Feresu, S.A., S.D. Harlow, and G.B. Woelk, Risk Factors for Low Birthweight in Zimbabwean Women: A Secondary Data Analysis. PLoS One, 2015. **10**(6): p. e0129705.

34. Ndibazza, J., et al., Effects of deworming during pregnancy on maternal and perinatal outcomes in Entebbe, Uganda: a randomized controlled trial. Clin Infect Dis, 2010. **50**(4): p. 531-40.

35. Shulman, C.E. and E.K. Dorman, Importance and prevention of malaria in pregnancy. Trans R Soc Trop Med Hyg, 2003. **97**(1): p. 30-5.

36. Muanda, F.T., et al., Antimalarial drugs for preventing malaria during pregnancy and the risk of low birth weight: a systematic review and meta-analysis of randomized and quasi-randomized trials. BMC Med, 2015. **13**: p. 193.

37. Liberati, A., et al., The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Ann Intern Med, 2009. **151**(4): p. W65-94.

38. Moher, D., et al., Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev, 2015. **4**: p. 1.

3.4 Research paper 1: Is birth weight associated with blood pressure among African children and adolescents? A systematic review

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	Abubaker Swaib Lule
Principal Supervisor	Emily Webb
Thesis Title	Investigating the impact of early-life, life-course and genetic factors on blood pressure among young Africans

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	Journal of Developmental Origins of Health and Disease		
When was the work published?	22/January/2018		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

**If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.*

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Developed the study protocol, performed the searches, retrieved papers, screened and assessed titles and abstracts, drafted, revised and submitted the manuscript to the journal

Student Signature: _____ **Date:** 02-10-2018

Supervisor Signature: Emily Webb **Date:** 2/10/2018

Journal of Developmental Origins of Health and Disease (2018), **9**(3), 270–280.

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REVIEW

Is birth weight associated with blood pressure among African children and adolescents? A systematic review

S. A. Lule[1,2]*, A. M. Elliott[1,2], L. Smeeth[1] and E. L. Webb[1]

[1]*London School of Hygiene and Tropical Medicine, London, UK*
[2]*MRC/UVRI Uganda Research Unit, Entebbe, Uganda*

There is substantial evidence of an inverse association between birth weight and later blood pressure (BP) in populations from high-income countries, but whether this applies in low-income countries, where causes of low birth weight are different, is not certain. Objective: We conducted a review of the evidence on the relationship between birth weight and BP among African children and adolescents. Medline, EMBASE, Global Health and Web of Science databases were searched for publications to October 2016. Papers reporting the relationship between birth weight and BP among African children and adolescents were assessed. Bibliographies were searched for further relevant publications. Selected papers were summarized following the preferred reporting items for systematic review and meta-analysis (PRISMA) guidelines. In total, 16 papers from 13 studies conducted in nine African countries (Nigeria, Republic of Seychelles, Gambia, Democratic Republic of Congo, Cameroon, South Africa, Algeria, Zimbabwe and Angola) were reviewed. Eight studies were cohorts, while five were cross-sectional. The relationship between birth weight and later BP varied with age of the participants. Studies in neonates showed a consistently positive association, while predominantly inverse associations were seen among children, and studies in adolescents were inconsistent. Based on the limited number of studies identified, the relationship between birth weight and later BP may vary with age in African children and adolescents. Not all studies adequately controlled for confounding, notably gender or age. Whether the inverse relationship between birth weight and BP in later life observed in Western settings is also seen in Africa remains unclear.

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Key words: Africa, birth weight, blood pressure, systematic review

Introduction

A strong geographical correlation between infant mortality (from 1921 to 1925) and adult ischaemic heart disease (IHD) mortality (from 1968 to 1978) was observed by Barker and Osmond.[1] They postulated that factors that increased the risk of death during infancy also increased susceptibility to IHD among those who survived infancy, and later, showed that blood pressure (BP) in adulthood was positively related to placenta weight but inversely associated with birth weight.[2] They suggested that poor fetal nutrition indicated by intrauterine growth restriction and low birth weight was associated with this increased susceptibility to IHD.

Subsequently, several studies [mainly from high-income countries (HICs)] have investigated the relationship between birth size parameters (e.g. birth weight, head circumference, placenta size) and later cardiovascular disease risk (mainly BP), with birth weight the most widely studied parameter. Results from several of these studies have shown an inverse association between birth weight and BP later in life.[3–7] A smaller number of studies have reported positive or no association between birth weight and later BP. For example, positive associations have been reported among UK neonates[8] and Chinese children,[9] whereas birth weight was not associated with BP among American adolescents.[10] The relationship between birth weight and later BP differed by gender among UK adolescents: a negative association was seen in the males but a positive association in the females.[11] Systematic reviews have reported that, on average, systolic blood pressure (SBP) drops by 2–4 mmHg for every kilogram increase in birth weight.[12,13] These reviews have predominantly comprised of studies among adults from HICs.

In HICs the prevalence of low birth weight varies between 5 and 8%.[14] Low birth weight is more common in Africa (7% in Nigeria,[15] 8% Uganda,[16] 11% Zambia,[17] 17% Zimbabwe and Benin[18,19] and 28% Ethiopia[18]) and on average African population have lower birth weights when compared with European populations.[20] In HICs, low birth weight is predominantly due to prematurity (most commonly as a result of maternal smoking in pregnancy[21]), whereas, in developing countries, low birth weight for gestational age constitutes most of the low birth weight infants.[22] The causes of low birth weight differ between rural, tropical Africa and developed or non-tropical settings: for example, malaria (an important cause of low birth weight) is restricted to the tropics[23] and prophylactic antimalarial drugs in pregnancy reduce the risk of low birth weight.[24,25] We hypothesized that the relationship between birth weight and BP in African settings might differ from that commonly observed in HICs.

*Address for correspondence: S. A. Lule, Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK.
(Email swaiblule@yahoo.com)

The role of birth weight in the later development of BP is important to African countries: these have a high burden of malnutrition,[26–28] low birth weight[18,19] and raised BP.[29–32] Early life interventions that reduce maternal malnutrition and extremes of birth weight (both low and high) may thus control childhood BP (before clinical manifestation of disease) and could be vital in the prevention of high BP in adulthood.

The absence of birth weight records for adults in many African countries and the low accuracy of maternally recalled birth weight[33] limits prospects for studying the relationship between birth weight and BP in adulthood in this setting. However, the emergence of a number of birth cohorts (with birth records) in Africa provides opportunities to investigate the relationship between birth weight and BP among African children and adolescents. Childhood BP predicts BP in early adulthood,[34,35] thus studies of the relationship between birth weight and BP among children are important in the identification of at-risk groups for targeted interventions early in life. We conducted a qualitative assessment of the direction and consistency of the relationship between birth weight and BP among African children and adolescents using a systematic review of existing literature.

Methods

A literature search covering publications up to 15 October 2016 with no restriction on start date was performed using Medline, EMBASE, Global Health and Web of Science databases. The search was performed on combinations of the keywords: (hypertension OR blood pressure) AND (birth weight) AND (paediatric OR child OR young people OR youth OR juvenile OR adolescent OR youngster OR pubescent OR teenage OR new-born OR minor OR infant) AND (Africa OR individual names of countries in Africa).

Original papers on the relationship between birth weight and BP among children and or adolescents, between ages 0 and 19 years and resident in Africa were reviewed. No restrictions on language or publication dates were applied. Publications on children and or adolescents of African ancestry not residing in Africa were excluded. Papers on the same participants were considered as one study. If more than one paper reported on the same participants at the same age, the most complete paper was included in the review. Papers reporting on the same participants were included and reviewed separately if they reported on the relationship between birth weight and BP at different ages. No additional information was sought from authors. Reference lists of the included papers were searched for additional relevant publications.

Search results were exported to Endnote reference management software (Thomson Reuters, version ×7) and duplicates removed. Two independent authors (S.L. and E.W.) assessed titles and abstracts for inclusion in the full-text review and then assessed full-text articles for inclusion in the data synthesis. Inconsistencies were discussed and consensus reached at each stage of the selection process.

Data were extracted independently by two authors (S.L. and E.W.) using standardized data extraction sheets on the year of publication, year of birth, location, age of participants, study design, number of participants, exclusion criteria, study aim, mean birth weight, source of birth weight data, BP measurement procedure, mean BP [SBP and, or, diastolic blood pressure (DBP)], relationship between birth weight and BP and how this was assessed, and whether there was adjustment for confounders. Information was recorded as presented in the original publication, except where the overall mean BP or birth weight was missing; in this case, where possible the overall mean was calculated from any stratum-specific means presented. Studies were assessed for selection bias and adjustment for confounding. Meta-analysis was not performed due to diversity in studies included in the review, in terms of their design, analysis, source population and covariates controlled for in the analysis. Guidelines from the preferred reporting items for systematic review and meta-analysis (PRISMA)[36] were followed.

Results

A total of 990 published abstracts were retrieved from four databases, of these 366 duplicates were removed, leaving 624 abstracts for review (Fig. 1). Of these, 562 were excluded and of the remaining 62 papers that were subjected to full-text review, 46 were excluded. Of the 46 papers excluded, two papers[37,38] were duplicates (reported on the same participants at the same age) of one of the included papers. Thus, 16 papers from 13 studies describing, but not necessarily focussing on, the relationship between birth weight and BP were included in the final review and data extraction (Fig. 1).

Of the 16 papers reviewed, six were from West Africa,[39–44] six Southern Africa,[45–50] two Central Africa,[51,52] one East Africa[53] and one North Africa.[54] Four papers from Southern Africa were from the same cohort but presented data on BP at different ages of follow-up.[46–49] Four papers reported results in neonates (0–28 days),[41,43,44,51] four in children (1–9 years),[39,40,49,55] four in adolescents (10–19 years)[42,47,48,54] and four in both children and adolescents.[45,46,52,53] The papers were published between 1989 and 2016.

The main characteristics of the reviewed papers are shown in Table 1. Briefly, all papers included both males and females. The number of participants ranged from 157 to 2743 individuals per paper, with five papers reporting on more than 1000 participants. Seven papers had less than 500 participants. Two of the reviewed papers did not present quantitative information on the relationship between birth weight and BP. Eleven of the papers (from eight studies) described results from cohorts, while five papers reported results from cross-sectional studies.

Except for one paper, in which the source of participants was unclear, participants were recruited from schools ($n = 5$),[45,50,52–54] hospitals ($n = 4$)[41,43,44,51] and communities ($n = 6$; representing three studies).[40,42,46–49] Preterm children were excluded in seven papers: all four of the hospital-based

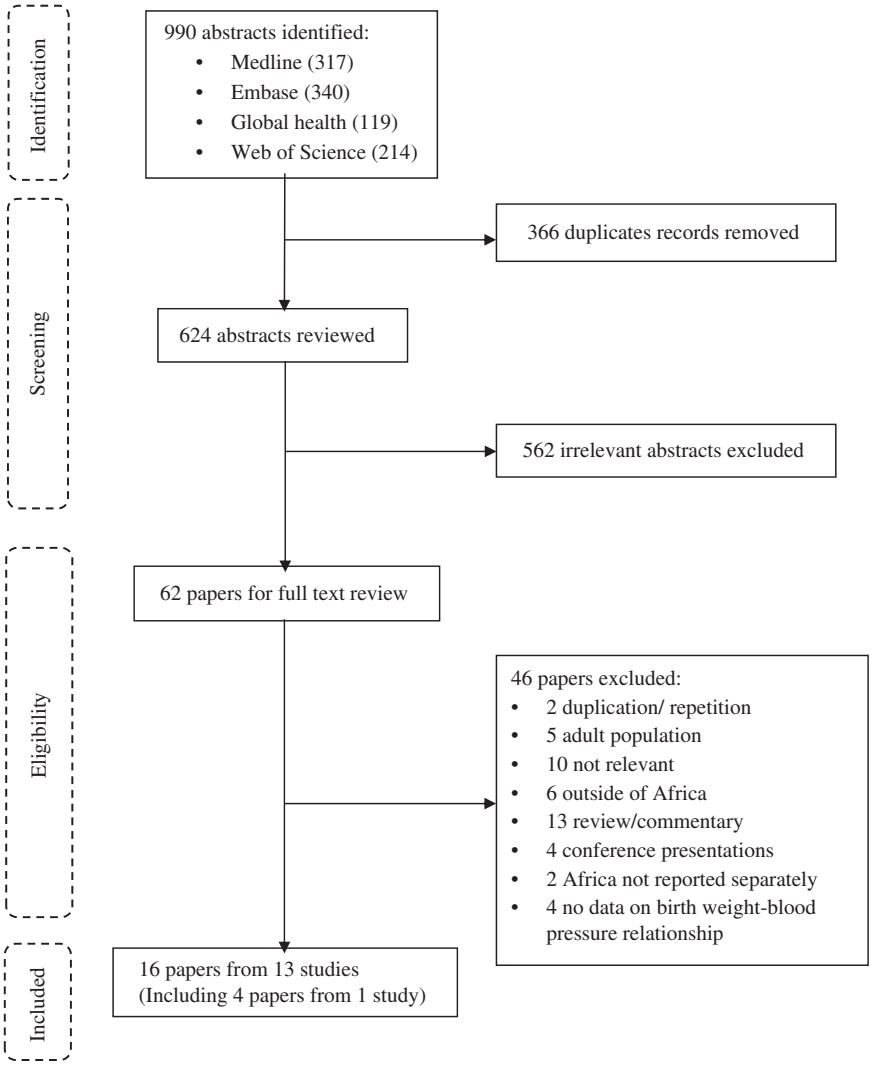

Fig. 1. Flow diagram for systematic review.

studies,[41,43,44,51] one school-based[52] and one community-based.[42] Twins were excluded in six papers (representing three studies).[41,46–48,50] Children who were small for gestational age or who weighed <2.5 kg at birth were excluded from three papers; one in neonates,[44] one in children[39] and one in children and adolescents.[52] There was variability in the study aims of the papers included: only six described assessing the association between birth weight and BP as one of their main aims.[39,50,52–54,56]

Table 2 summarizes birth weight and BP measurements and values, statistical analysis methods and the relationship between birth weight and BP in the reviewed papers.

Birth weight ascertainment

Birth weight was either measured and recorded immediately after birth (predominantly in the cohort studies), or extracted from birth or child health card records. In one study,[52] parentally recalled birth weight was used when birth records were missing (in an unknown number of participants). Mean birth weight varied from 2.4 to 3.4 kg.

BP assessment

BP procedures were relatively similar across studies. All studies used automated devices, except for one study, which used the sphygmomanometer machine. Eleven papers reported a resting period (from 5 to 20 min) before proceeding with measuring the BP. In four studies, measurements were taken on the left arm, whereas in six studies measurements were on the right arm; the remaining studies did not include this information.

BP was measured in triplicates in the majority of papers ($n = 12$) with one paper reporting single measurement, two reporting double measurement and one reporting five measurements. The rest period between consecutive BP measurements varied from 1 to 3 min.

Of the 12 papers that measured BP in triplicate, five used the mean of all three measurements in data analysis, five used the

Table 1. *Description of the studies included in the systematic review*

Authors	Year of publication	Year of birth	Place, country	Age at BP assessment	Study size	Study type	Source of subjects	Reason for exclusion	Study aim
Ayoola	2011	NM	Ibadan, Nigeria	1–3 days	436	Cohort	Hospital	Preterm, twins, metabolic defects, congenital abnormalities or sever birth trauma, babies of women with HIV, STDs, hypertension or diabetes	Evaluate the impact of maternal malaria on new-born BP
Chiolero	2011	1984–1997	Republic of Seychelles	5.5, 9.1, 12.5, 15.5 years	2743	Cohort	School	Not mentioned	Assess the association between BW, weight change, and current BP across the entire age span of childhood and adolescence
Hawkesworth	2009	1989–1994	West Kiang, Gambia	11–17 years	1267	Cohort	Community	Preterm, implausible BP reading, ambiguity on treatment allocation	Investigate the effect of maternal protein-energy supplementation on BP in adolescence
Longo-Mbenza	1999	1980–1991	Kinshasa, DRC	5–16 years, mean 11 years	2409	CS	School	Preterm birth, small for gestational age	Examine the possible association between LBW and hypertension later in life
Margetts	1991	1980–1988	Rural, Gambia	1–9 years	675	Cohort	Community	Febrile illnesses	Relate BP levels in children to their mother's weight in pregnancy
Sadoh	2010	NM	Benin City, Nigeria	1–4 days	473	CS	Hospital	Preterm, abnormal APGAR score, congenital abnormality, admission to neonatal unit, babies of mothers with pre-eclampsia or diabetes	Determine the association between maternal and neonatal factors with BP at birth
Salvi	2010	1986–1990	Metlili, Algeria	15–19 years	568	CS	School	Major disabilities, significant heart disease, renal or liver disease	Assess the association of current body weight and birth weight with BP values in school children living in Algerian Sahara
Law	2000	1989–1993	Sagamu Nigeria	3–6 years, mean 4.4 years	293	Cohort	NM	Preterm, weighing less than 2.5 kg	Determine how reduced fetal growth is related to raised BP in countries where chronic malnutrition is common
Woelk	1998	1987–1989	Harare, Zimbabwe	Mean 6.5 years	583	Cohort	School	Twins, not born in Harare	Determine whether poor uterine growth may be associated with increased BP and subsequent hypertension in adulthood

Table 1: (*Continued*)

Authors	Year of publication	Year of birth	Place, country	Age at BP assessment	Study size	Study type	Source of subjects	Reason for exclusion	Study aim
Silva	2016	2000–2006	Luanda, Angola	7–12 years, mean 9.4 years	157	CS	School	Not classified as Tanner stage I, completed 12 months between recruitment and examinations, high BP, obesity	Determine the factors associated with pulse wave velocity values and propose preliminary reference values in pre-pubertal Angolan school children
Nwokoye	2015	NM	Enugu, Nigeria	1–2 days	310	CS	Hospital	Birth asphyxia, preterm, postterm, sick babies, not weighing 2.5–4.0 kg, babies of mothers on antihypertensive drugs or illicit drugs	Determine BP values in apparently healthy term new borns in the first 48 h of life and evaluate the factors affecting BP at birth
Youmbissi	1989	NM	Yaounde, Cameroon	At birth	202	Cohort	Hospital	Preterm, sick neonates, babies of mothers on diuretics or antihypertensive therapy during pregnancy or labour	Evaluate SBP variations in new borns
Levitt[a]	1999	1990	Soweto, South Africa	5 years	818	Cohort	Community	Twins	Examine the relationship between BW and BP at 5 years in a cohort of South African children
Kagura[a]	2016	1990	Soweto South Africa	5, 8, 10, 13, 14, 16 and 18 years	1937	Cohort	Community	Twins, non-black, pregnancy during adolescence	Examine the association between early growth and BP trajectories and assess the influence of height on the association between early growth and BP trajectories
Griffiths[a]	2012	1990	Soweto, South Africa	16 years	358	Cohort	Community	Twins, non-black	Understand the relationship between household and neighbourhood SES with SBP
Adair[a]	2013	1990	Soweto, South Africa	18 years	1222	Cohort	Community	Twins	Investigate how linear growth and weight gain relative to linear growth in childhood and adulthood are related to health and human capital outcomes in young adults

BP, blood pressure; BW, birth weight; DRC, Democratic Republic of Congo; CS, cross-sectional study; LBW, low birth weight; NM, not mentioned; SBP, systolic blood pressure; SES, socioeconomic status.

[a]Papers from the same cohort (Birth to Twenty cohort).

Table 2. *Main results from the studies included in the systematic review*

Authors	Mean BW	Source of BW data	Mean BP (mmHg)	Procedure for BP measurement	Relationship between BW and BP	Adjusting factors
Ayoola	2.9 kg	Hospital birth records	SBP = 71.0 DBP = 36.1	With the child comfortable on the mother's lap for 5 min or asleep, three BP measurements with a minute's interval between successive measurements were taken on the left arm using a Datascope monitor. Mean of the last two readings was used for analysis	Positive BW–BP association. SBP increased by 8.35 mmHg/kg increase in BW (95% CI: 4.36, 12.35, $P < 0.001$). DBP increased by 3.07 mmHg/kg increase in BW (95% CI: 0.26, 5.88, $P = 0.032$)	Gestational age, baby length, maternal malaria, age, weight, height, BP, gravidity, antenatal visits
Chiolero	3.1 kg	Medical records	Could not be determined	After 5-min rest, two BP measurements were taken on the right arm with a minute's interval between each using automated devices (OmronM5; Omron, UK). Mean of the two measurements was used for analysis	No overall BW–BP association. BW was not associated with SBP or with DBP, with exception of girls at 12.5 years among whom BW was inversely associated with SBP ($\beta = 0.9$, 95% CI: −1.6, −0.1, $P = 0.026$) and DBP ($\beta = -0.7$, 95% CI: −1.3, −0.1, $P = 0.028$)	Current weight
Hawkesworth	2.9 kg	Birth records	SBP = 110.5 DBP = 64.7	Measured in triplicate using the automated Omron 7051 T device (Omron), following manufacturer's instruction. Mean of the three measurements was used for analysis	No association between BW and BP, $\beta = -0.001$, 95% CI: −0.002, 0.000, $P = 0.06$	Age, current body size, sex, gestational age, birth season
Longo-Mbenza	2.4 kg	Parental recall, medical records	Could not be determined	With child in a sitting position and relaxed for 20 min, five BP measurements were obtained using an automatic device (HEM-705 CP; Omron, Tokyo, Japan). Not clear which measurements were used in the analysis	BW inversely correlated with BP. With $r = -0.1$, $P < 0.001$ for SBP and $r = -0.1$, $P < 0.05$ for DBP. LBW had twice the odds of hypertension OR = 2.0, 95% CI: 0.9, 8.2, $P < 0.01$ for SBP and OR = 2.3, 95% CI: 0.6, −11.5, $P < 0.01$ for DBP[a]	NM
Margetts	3.0 kg	Birth records	1 year: SBP = 89.3 DBP = 56.2, 9 years: SBP = 102.7 DBP = 63.9	After 5 min in a sitting position or on the mother's knee in the young children, BP was measured twice on the right arm using an automated device (Dinamap model: 18465X). Not clear which measurements were used in the analysis	BW not associated with SBP at any age	NM
Sadoh	3.2 kg	Hospital birth records	SBP = 69.2	Measured 1 h after feeds between 11:00 and 13:30 using a Dinamap 8100 monitor (Critikon, Tampa Fla) device, when the baby was asleep or awake and calm. Three BP measurements were obtained within 3 min of each other on the right arm. Mean of three BP readings was used in the analysis	Positive BW–BP association. BW was correlated with SBP ($r = 0.235$, $P = 0.001$), SBP rose by 3.61 mmHg/0.5 kg increase in birth weight	NM
Salvi	3.4 kg	Obstetric records in local hospitals	SBP = 118.1 DBP = 69.9	Three BP measurements with 3-min interval between successive measurements were taken on the left arm, after 10-min rest in a sitting position. An automated oscillmetric device (Omron 705 T; Omron) was used. Average of the three measurements was used for the analysis	No correlation between BW and SBP	Multivariate analysis was not done
Law	3.2 kg	Birth records	SBP = 101.6	After 5-min rest, three BP measurements were taken on the left arm using automated BP machines (Dinamap model 8100) with a 1-min interval between consecutive measurements. Mean of the three measurements was used for the analysis	No association between BW and SBP ($\beta = 0.4$, 95% CI: −2.1, 2.9)	Gender, observer, child's status, (crying or not) current weight and cuff size

Table 2: (*Continued*)

Authors	Mean BW	Source of BW data	Mean BP (mmHg)	Procedure for BP measurement	Relationship between BW and BP	Adjusting factors
Woelk	3.0 kg	Birth records	SBP = 108.3 DBP = 62.1	With child sitting quietly, BP was measured in the morning on the right arm. Three measurements were taken 2-min apart using a Dinamap model 8100 BP. The average of the last two BP readings was used for analysis	Inverse relationship between BW and SBP. SBP rose by 1.73 mmHg/kg decrease in BW (95% CI: 0.18, 3.28), $P = 0.0286$. No association between BW and DBP ($\beta = -1.06$, 95% CI: −2.57, 0.45)	Current weight
Silva	3.2 kg	–	SBP = 102 DBP = 62	After resting for 5–10 min in a sitting position, three consecutive BP measurements were taken on the left arm with a 2-min interval using an automatic sphygmomanometer (model HEM-742; OMRON, Nanjing, China). Mean of last two readings was used for analysis	No correlation between BW and SBP, $r = -0.016$, weak correction between DBP and BW $r = 0.09$, $P < 0.05$. Categorizing birth weight into four groups, no association between birth weight category and SBP ($P = 0.991$) or DBP ($P = 0.059$)	NM
Nwokoye[b]	2.5–4.0 kg	Hospital birth records	Day 1, SBP = 63.3 DBP = 36.8 Day 2, SBP = 65.6 DBP = 40.0	After 10–15-min rest, a single BP measurement was taken on the right arm when the infant was awake and quiet or asleep and in spine position using an oscillometic machine (Dinamap 8100)	Positive correlation between BW and SBP, $r = 0.37$, $P < 0.01$ between 0–24 h and $r = 0.29$, $P < 0.01$ between 25–48 h. No correlation between BW and DBP	NM
Youmbissi	3.2 kg	Hospital birth records	SBP = 65.1	Measured in the morning, on the right arm of a quiet and awake child. Three measurements were taken using a zero sphygmomanometer. Average of the three measurements used for the analysis	No association between BW and SBP, $r = 0.12$	NM
Levitt[c]	3.1 kg	Birth records	SBP = 108.0 DBP = 62.6	After 10-min rest, BP was measured in triplicate using a Dinamap vital signs Monitor (1846SX). The lowest DBP with its matching SBP were used in the analysis	Inverse association between BW and SBP ($r = -0.05$, $P < 0.001$), SBP fell by 3.4 mmHg, (95% CI: 1.4, 5.3)/kg increase in BW. No association between BW and DBP	Current weight and height
Kagura[c]	3.1 kg	Birth records	Could not be determined	After 5-min rest, BP was measured in triplicate with 2-min intervals between successive measurements, using the Dinamap Signs monitor 1846SX (Critikon) at 5 years and Omron M6 (Omron) at 8–18 years. Not clear which measurements were used in the analysis	Inverse association between BW and middle BP trajectory among boys (OR = 0.75, 95% CI: 0.58, 0.96, $P = 0.0223$). No other associations seen	Height, SES, maternal age, parity and gestational age
Griffiths[c]	3.1 kg	Birth records	SBP = 114.8	With participants in a sitting position, three measurements were taken using a digital (Omron M6; Omron) device with a rest of several minutes between successive measurements. The average of the last two measurements was used for analysis	Inverse association between BW and SBP among boys ($\beta = -0.003$, $P < 0.1$). No association between BW and SBP among girls	Current height
Adair[c]	3.1 kg	Hospital birth records	SBP = 117.5 DBP = 71.4	After 5–10-min rest, three BP measurements were taken using a digital device (Omron M6). The average of the last two measurements was used for analysis	No association between BW and BP. For SBP $\beta = 0.05$, 95% CI: −0.79, 0.90 in males and 0.08 (−0.69, 0.84) in females, and for DBP $\beta = -0.05$ (−0.78, 0.68) among males and 0.17 (−0.51, 0.85) in females	NM

BW, birth weight; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; CI, confidence interval; LBW, low birth weight; OR, odds ratio; NM, not mentioned; SES, socioeconomic status; *r*, correlation coefficient; β, linear regression coefficient.

[a]95% CI and *P*-value reported as in paper but are inconsistent.

[b]Mean birth weight not reported and could not be calculated (determined).

[c]Papers from the same study group (Birth to Twenty cohort).

mean of the last two measurements, one used the lowest DBP (with matching SBP) and one was unclear. For the paper where BP was measured five times and one of the two papers where BP was measured in duplicate, it was unclear which measurements or combination thereof were used for data analysis. In the other paper where BP was measured in duplicate, the mean of the two measurements was used in the analysis.

Among neonates, mean SBP varied from 65.1 to 71.0 mmHg and in children and adolescents from 89.3 to 118.1 mmHg. Mean DBP varied from 36.1 to 63.9 mmHg in neonates and between 56.2 and 71.4 mmHg among children and adolescents. Mean SBP and DBP generally increased with age over the course of childhood and adolescence, this was especially apparent in the four papers that reported results from the same cohort study at different ages.

Birth weight and BP relationship

The relationship between birth weight and SBP varied across papers; seven papers reported no association,[39,40,42,45,47,51,54] six an inverse association[46,48–50,52,53] and three a positive association.[41,43,44] Among the neonates, three out of four papers reported a positive association while one reported no association. Of the four papers in children, two reported inverse associations and two no association. The papers on children and adolescents predominately found inverse associations (three out of four papers) while among adolescents, three papers found no association and one an inverse association.

Of the seven papers with participant size less than 500 individuals,[39,41,43–45,48,51] three papers reported no association between birth weight and SBP, one an inverse association and three a positive association.[43–45,51] Studies with larger participant sizes (greater than 500 individuals) were more likely to report inverse associations. Of the nine studies with participant size over 500, three reported no association between birth weight and SBP, five an inverse association and one reported a positive association.

In three of the six papers reporting inverse associations,[46,48,53] analyses were conducted at different ages and, or, separately for males and females, with inverse associations only seen among particular subgroups (girls at 12.5 years,[53] boys only[46,48]) and analyses from other subgroups showing no evidence of association.

Analysis approaches used to assess the relationship between birth weight and BP were diverse, varying from simple correlation analysis with no adjustment for potential confounders, to more complex group-based trajectory modelling approaches. Multivariable analysis, adjusting for potential confounder(s) [often including age, sex or body size (weight or height)] was conducted in eight papers, of these five reported an inverse association, two no association and one a positive association. In comparison, of the eight papers that did not undertake adjustment for confounders, one reported an inverse association between birth weight and SBP, five reported no association and two a positive association.

The relationship between birth weight and DBP was described in eight papers; a positive association was seen in two papers, inverse association in two papers and no association was reported in four papers. Of these eight papers, two were in neonates, two in children, three in children and adolescents, and one in adolescents only.

Discussion

Overall, this systematic review of existing literature showed varied results. We identified 16 papers from 13 studies addressing the question of whether the inverse relationship between birth weight and BP in later life seen in Western settings is also present in Africa. The relatively small number of studies and their heterogeneity in design and analysis prohibits definitive conclusions. However, we found some evidence to suggest that the relationship between birth weight and SBP in Africa varies with the participants' age: positive associations were seen in neonates and inverse associations mainly in children. Among adolescents, the relationship was either inverse or showed no evidence of association. Only a few papers reported on the relationship between birth weight and DBP, with most papers reporting no relationship between birth weight and DBP.

This review supports an earlier review by Law[12] that did not include any of the papers reviewed herein (only two of the papers included in the present review had been published at the time of the Law review, and of these Margetts *et al.*[40] was excluded for missing quantitative information while Youmbissi *et al.*[51] was not mentioned). The Law review reported inconsistencies in the relationship between birth weight and BP, especially among adolescents. Generally, inverse associations were among children and positive associations in neonates, inconsistencies could be due to differences in age, sample sizes and statistical analysis approaches. Interestingly, results from the cohort studies included in our review, that measured BP at more than one-time point (different ages), did not show evidence of increasing strength of the association between birth weight and BP with age as reported by the earlier review.[12] Studies in neonates consisted mainly of less than 500 participants and reported positive associations. Studies with smaller participant numbers are more likely to be underpowered to detect real associations, but also to produce spurious positive or negative associations.[57] However, this may not be a factor for the results among neonates, which are consistent.

The relationship between birth weight and BP among adolescents has been reported in previous reviews as either inconsistent[12] or inverse with smaller effects than observed among (prepubescent) children.[13] Similarly, this review found an inconsistent relationship between birth weight and BP among adolescents, while the relationship among younger children was generally inverse. The positive relationship observed in neonates is as expected, with the duration between birth and BP assessment too short to allow for any impact of subsequent weight trajectory. Explanations for the changing relationships

among children and adolescents are uncertain, but could possibly relate to different growth patterns, for example, catch-up growth among those of low birth weight, and hormonal changes occurring at adolescence.[12,58]

Adjustment for possible confounding factors varied and was often incomplete. Consistent with previous reviews, which mainly included papers from HICs, studies adjusting for current body size (weight and, or, height) were more likely to report an inverse relationship[59,60] than those that did not make such an adjustment. Adjusting for current size has been noted to lead to a stronger inverse relationship between birth weight and BP compared with results without such adjustments.[6] In most of the reviewed papers, that reported estimates adjusted for current size, unadjusted estimates for the effect of birth weight on BP were not reported. Therefore, we were unable to establish whether adjusting for current weight leads to stronger inverse relationships in these populations. The interpretation of findings adjusting for current weight is complex[6,59] because current weight may be seen as a confounder or mediator of the effect of birth weight on BP.[6]

Several mechanisms such as obesity, salt-sensitivity, renin–angiotensin system and endothelial activation are important in the pathophysiology of hypertension. None of the reviewed papers investigated the role of these factors and their impact on BP. Recent evidence suggests that the relationship between birth weight and BP could be U shaped,[61] highlighting the importance of both reduced and excessive nutrition in utero. It was not possible to examine this hypothesis from the papers reviewed. Compared with birth weight, other measures such as birth body mass index or pendular index may more accurately reflect the birth size, but none of the studies reviewed included these measures.

Studies were subject to selection bias as individuals most likely to be low birth weight (such as preterm and twins) were excluded in many studies. This could have led to an underestimation of the effect of birth weight on BP. Furthermore, characteristics [such as maternal hypertension, parasitic infections, socioeconomic status (past or current)] that influence birth weight and may also be associated with BP in offspring were not adjusted for in these studies. Hence, estimates were subject to residual confounding. It remains uncertain what role (if any) such factors have in the relationship between birth weight and subsequent BP in children or adolescents.

Inconsistencies seen between reviewed papers are less likely to be due to differences in BP measurement procedures, as studies followed a similar approach. For nearly all papers, there was an initial rest period (before starting BP procedure) and between successive BP measurements, automated devices were used and analysis was based on the average of two or three measurements. In the majority of studies, early life information including birth weight was prospectively collected thus studies were less prone to misclassification and recall bias.

Generally, studies reviewed came from all the African regions but with a strong representation of West Africa and Southern Africa. East Africa and North Africa had the least number of papers (one each) reviewed. The only paper from East Africa reported on an island population, thus there were no results on the mainland population of East Africa.

In conclusion, relatively few studies have investigated the relationship between birth weight and BP later in life in Africa. The relationship between birth weight and BP varied depending on the age of the participants. Our review emphasizes the need for larger studies on the relationship between birth weight and later BP from Africa, applying appropriate control of potential confounding factors. Accumulating evidence on raised BP in Africa and understanding the impact of growth in early life and of prenatal exposures on BP later in life is key in identifying at-risk groups and developing early life interventions to reduce BP risk in later life.

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Ethical Standards

Review was based on published manuscripts thus ethical approvals were not required.

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Conflicts of Interest

None.

References

1. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet*. 1986; 1, 1077–1081.
2. Barker DJ, Bull AR, Osmond C, Simmonds SJ. Fetal and placental size and risk of hypertension in adult life. *BMJ*. 1990; 301, 259–262.
3. Schack-Nielsen L, Holst C, Sorensen TI. Blood pressure in relation to relative weight at birth through childhood and youth in obese and non-obese adult men. *Int J Obes Relat Metab Disord*. 2002; 26, 1539–1546.
4. Adair LS, Martorell R, Stein AD, *et al*. Size at birth, weight gain in infancy and childhood, and adult blood pressure in 5 low- and middle-income-country cohorts: when does weight gain matter? *Am J Clin Nutr*. 2009; 89, 1383–1392.
5. Law CM, de Swiet M, Osmond C, *et al*. Initiation of hypertension in utero and its amplification throughout life. *BMJ*. 1993; 306, 24–27.
6. Blake KV, Gurrin LC, Evans SF, *et al*. Adjustment for current weight and the relationship between birth weight and blood pressure in childhood. *J Hypertens*. 2000; 18, 1007–1012.

7. Pharoah PO, Stevenson CJ, West CR. Association of blood pressure in adolescence with birthweight. *Arch Dis Child Fetal Neonatal Ed*. 1998; 79, F114–F118.
8. O'Sullivan MJ, Kearney PJ, Crowley MJ. The influence of some perinatal variables on neonatal blood pressure. *Acta Paediatr*. 1996; 85, 849–853.
9. Perng W, Rifas-Shiman SL, Kramer MS, *et al*. Early weight gain, linear growth, and mid-childhood blood pressure: a prospective study in project viva. *Hypertension*. 2016; 67, 301–308.
10. Falkner B, Hulman S, Kushner H. Effect of birth weight on blood pressure and body size in early adolescence. *Hypertension*. 2004; 43, 203–207.
11. Macintyre S, Watt G, West P, Ecob R. Correlates of blood pressure in 15 year olds in the west of Scotland. *J Epidemiol Community Health*. 1991; 45, 143–147.
12. Law CM, Shiell AW. Is blood pressure inversely related to birth weight? The strength of evidence from a systematic review of the literature. *J Hypertens*. 1996; 14, 935–941.
13. Huxley RR, Shiell AW, Law CM. The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. *J Hypertens*. 2000; 18, 815–831.
14. Kim D, Saada A. The social determinants of infant mortality and birth outcomes in Western developed nations: a cross-country systematic review. *Int J Environ Res Public Health*. 2013; 10, 2296–2335.
15. Dahlui M, Azahar N, Oche OM, Aziz NA. Risk factors for low birth weight in Nigeria: evidence from the 2013 Nigeria Demographic and Health Survey. *Glob Health Action*. 2016; 9, 28822.
16. Ndibazza J, Muhangi L, Akishule D, *et al*. Effects of deworming during pregnancy on maternal and perinatal outcomes in Entebbe, Uganda: a randomized controlled trial. *Clin Infect Dis*. 2010; 50, 531–540.
17. Chibwesha CJ, Zanolini A, Smid M, *et al*. Predictors and outcomes of low birth weight in Lusaka, Zambia. *Int J Gynaecol Obstet*. 2016; 134, 309–314.
18. Assefa N, Berhane Y, Worku A. Wealth status, mid upper arm circumference (MUAC) and antenatal care (ANC) are determinants for low birth weight in Kersa, Ethiopia. *PloS One*. 2012; 7, e39957.
19. Denoeud L, Fievet N, Aubouy A, *et al*. Is chloroquine chemoprophylaxis still effective to prevent low birth weight? Results of a study in Benin. *Malar J*. 2007; 6, 27.
20. Shiono PH, Klebanoff MA, Graubard BI, Berendes HW, Rhoads GG. Birth weight among women of different ethnic groups. *JAMA*. 1986; 255, 48–52.
21. Delnord M, Blondel B, Zeitlin J. What contributes to disparities in the preterm birth rate in European countries? *Curr Opin Obstet Gynecol*. 2015; 27, 133–142.
22. Garner P, Kramer MS, Chalmers I. Might efforts to increase birthweight in undernourished women do more harm than good? *Lancet*. 1992; 340, 1021–1023.
23. Shulman CE, Dorman EK. Importance and prevention of malaria in pregnancy. *Trans R Soc Trop Med Hyg*. 2003; 97, 30–35.
24. Muanda FT, Chaabane S, Boukhris T, *et al*. Antimalarial drugs for preventing malaria during pregnancy and the risk of low birth weight: a systematic review and meta-analysis of randomized and quasi-randomized trials. *BMC Med*. 2015; 13, 193.
25. Cot M, Le Hesran JY, Miailhes P, *et al*. Increase of birth weight following chloroquine chemoprophylaxis during the first pregnancy: results of a randomized trial in Cameroon. *Am J Trop Med Hyg*. 1995; 53, 581–585.
26. Akombi BJ, Agho KE, Merom D, Renzaho AM, Hall JJ. Child malnutrition in sub-Saharan Africa: a meta-analysis of demographic and health surveys (2006-2016). *PloS One*. 2017; 12, e0177338.
27. Vorster HH, Kruger A. Poverty, malnutrition, underdevelopment and cardiovascular disease: a South African perspective. *Cardiovasc J Afr*. 2007; 18, 321–324.
28. Benzekri NA, Sambou J, Diaw B, *et al*. High prevalence of severe food insecurity and malnutrition among HIV-infected adults in Senegal, West Africa. *PloS One*. 2015; 10, e0141819.
29. Guwatudde D, Mutungi G, Wesonga R, *et al*. The epidemiology of hypertension in Uganda: findings from the national non-communicable diseases risk factor survey. *PloS One*. 2015; 10, e0138991.
30. Kavishe B, Biraro S, Baisley K, *et al*. High prevalence of hypertension and of risk factors for non-communicable diseases (NCDs): a population based cross-sectional survey of NCDS and HIV infection in Northwestern Tanzania and Southern Uganda. *BMC Med*. 2015; 13, 126.
31. Ataklte F, Erqou S, Kaptoge S, *et al*. Burden of undiagnosed hypertension in sub-Saharan Africa: a systematic review and meta-analysis. *Hypertension*. 2015; 65, 291–298.
32. Reddy SP, Mbewu AD. The Implications of the Developmental Origins of Health and Disease on Public Health Policy and Health Promotion in South Africa. Parthasarathy S, ed. *Healthcare*. 2016; 4, 83.
33. Lule SA, Webb EL, Ndibazza J, *et al*. Maternal recall of birthweight and birth size in Entebbe, Uganda. *Trop Med Int Health*. 2012; 17, 1465–1469.
34. Kagura J, Adair LS, Musa MG, Pettifor JM, Norris SA. Blood pressure tracking in urban black South African children: Birth to Twenty cohort. *BMC Pediatr*. 2015; 15, 78.
35. Hao G, Wang X, Treiber FA, *et al*. Blood pressure trajectories from childhood to young adulthood associated with cardiovascular risk: results from the 23-year longitudinal Georgia stress and heart study. *Hypertension*. 2017; 69, 435–442.
36. Moher D, Shamseer L, Clarke M, *et al*. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev*. 2015; 4, 1.
37. Steyn K, de Wet T, Richter L, *et al*. Cardiovascular disease risk factors in 5-year-old urban South African children – the birth to ten study. *S Afr Med J*. 2000; 90, 719–726.
38. Sadoh WE, Ibhanesebhor SE. Oscillometric blood pressure reference values of African full-term neonates in their first days postpartum. *Cardiovasc J Afr*. 2009; 20, 344–347.
39. Law CM, Egger P, Dada O, *et al*. Body size at birth and blood pressure among children in developing countries. *Int J Epidemiol*. 2000; 30, 52–57.
40. Margetts BM, Rowland MG, Foord FA, *et al*. The relation of maternal weight to the blood pressures of Gambian children. *Int J Epidemiol*. 1991; 20, 938–943.
41. Ayoola OO, Gemmell I, Omotade OO, *et al*. Maternal malaria, birth size and blood pressure in Nigerian newborns: insights into the developmental origins of hypertension from the Ibadan growth cohort. *PloS One*. 2011; 6, e24548.
42. Hawkesworth S, Prentice AM, Fulford AJ, Moore SE. Maternal protein-energy supplementation does not affect adolescent blood pressure in the Gambia. *Int J Epidemiol*. 2009; 38, 119–127.

43. Sadoh WE, Ibhanesehbor SE, Monguno AM, Gubler DJ. Predictors of newborn systolic blood pressure. *West Afr J Med*. 2010; 29, 86–90.
44. Nwokoye I, Uleanya N, Ibeziako N, *et al*. Blood pressure values in healthy term newborns at a tertiary health facility in Enugu, Nigeria. *Niger J Clin Pract*. 2015; 18, 584–588.
45. Silva ABT, Capingana DP, Magalhaes P, *et al*. Predictors and reference values of pulse wave velocity in prepubertal Angolan children. *J Clin Hypertens*. 2016; 18, 725–732.
46. Kagura J, Adair LS, Munthali RJ, Pettifor JM, Norris SA. Association between early life growth and blood pressure trajectories in black South African children. *Hypertension*. 2016; 68, 1123–1131.
47. Adair LS, Fall CH, Osmond C, *et al*. Associations of linear growth and relative weight gain during early life with adult health and human capital in countries of low and middle income: findings from five birth cohort studies. *Lancet*. 2013; 382, 525–534.
48. Griffiths PL, Sheppard ZA, Johnson W, *et al*. Associations between household and neighbourhood socioeconomic status and systolic blood pressure among urban South African adolescents. *J Biosoc Sci*. 2012; 44, 433–458.
49. Levitt NS, Steyn K, De Wet T, *et al*. An inverse relation between blood pressure and birth weight among 5 year old children from Soweto, South Africa. *J Epidemiol Community Health*. 1999; 53, 264–268.
50. Woelk G, Emanuel I, Weiss NS, Psaty BM. Birthweight and blood pressure among children in Harare, Zimbabwe. *Arch Dis Child Fetal Neonatal Ed*. 1998; 79, F119–F122.
51. Youmbissi TJ, Oudou N, Mbede J, Nasah BT. Blood pressure profiles of a group of African children in the first year of life. *J Trop Pediatr*. 1989; 35, 245–246.
52. Longo-Mbenza B, Ngiyulu R, Bayekula M, *et al*. Low birth weight and risk of hypertension in African school children. *J Cardiovasc Risk*. 1999; 6, 311–314.
53. Chiolero A, Paradis G, Madeleine G, *et al*. Birth weight, weight change, and blood pressure during childhood and adolescence: a school-based multiple cohort study. *J Hypertens*. 2011; 29, 1871–1879.
54. Salvi P, Meriem C, Temmar M, *et al*. Association of current weight and birth weight with blood pressure levels in Saharan and European teenager populations. *Am J Hypertens*. 2010; 23, 379–386.
55. Woelk GB. Is low birth weight a risk factor for adult hypertension? A literature review with particular reference to Africa. *S Afr Med J*. 1995; 85(Pt 2), 1348–1349, 1352–1353.
56. Levitt NS, Lambert EV, Woods D, *et al*. Impaired glucose tolerance and elevated blood pressure in low birth weight, nonobese, young South African adults: early programming of cortisol axis. *J Clin Endocrinol Metabol*. 2000; 85, 4611–4618.
57. Berlin JA, Begg CB, Louis TA. An assessment of publication bias using a sample of published clinical trials. *J Am Stat Assoc*. 1989; 84, 381–392.
58. Ewald DR, Haldeman DL. Risk factors in adolescent hypertension. *Glob Pediatr Health*. 2016; 3, 2333794X15625159.
59. Hardy R, Sovio U, King VJ, *et al*. Birthweight and blood pressure in five European birth cohort studies: an investigation of confounding factors. *Eur J Public Health*. 2006; 16, 21–30.
60. Huxley R, Neil A, Collins R. Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? *Lancet*. 2002; 360, 659–665.
61. Curhan GC, Willett WC, Rimm EB, *et al*. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. *Circulation*. 1996; 94, 3246–3250.

Chapter 4: Methods

4.1 Introduction

This chapter defines and classifies the terms used in the thesis, and also describes in detail the methods for the longitudinal observational study used to address thesis objectives 2, 3, 4 and 5. Statistical methods for each of the thesis objectives 2, 3, 4 and 5 are described in detail in the data analysis plan presented later in section 4.6 and in specific published research papers 2, 3 and 4 presented in respective chapters 5 to 7.

4.2 Definitions and classification

4.2.1 Blood pressure

In children and adolescents (0 to 18 years of age), BP classification is based on the normative distribution of BP and interpreted on the basis of BP percentile specific for sex, age and height. This thesis uses BP classifications and thresholds based on international criteria (the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents [1] and the 2009 guidelines by the European Society of Hypertension [2]).

- Hypertension: average systolic and/or diastolic BP ≥95th percentile for sex, age and height based on readings done on three or more separate occasions. Hypertension severity is staged as:
  - Stage 1 hypertension: average systolic or diastolic BP between 95th and 99th percentile plus 5 mmHg
  - Stage 2 hypertension: average systolic or diastolic BP >99th percentile plus 5 mmHg
- Pre-hypertension: average systolic BP and/or diastolic BP ≥90th percentile but <95th percentile for sex, age, and height based on measurements done on three or more separate occasions
- Normal BP: average systolic and diastolic BP levels <90th percentiles for sex, age and height based on measurements done on three or more separate occasions

Sine no standards exist for young African, this PhD used the USA standardised BP thresholds [3] to dichotomise individuals into pre-hypertensive, hypertensive and normal in this African population, thus allowed for easier comparison of pre-hypertension and hypertension prevalence from different studies or populations. The prevalence of high BP

in the population under study was anticipated to be low, thus data analysis was planned to use continuous BP values, allowing for more powerful and meaningful analysis than using binary categories of hypertensive and normal.

4.2.2 Birth weight

- Birth weight: body weight of a new-born measured in the first 72 hours [4]. Birth weight is categorised into:
  - Low birth weight: weight <2,500g irrespective of gestational age
  - Normal birth weight: weight ≥2,500g but <4,000g
  - High birth weight: weight ≥4,000g

The duration of gestation and or intrauterine growth rate determine birth weight, thus low birth weight may be due to short gestation (prematurity, also known as preterm) and or IUGR and or SGA [5].

- Prematurity: birth occurring before completion of 37 weeks of gestation [6].
- Intrauterine growth restriction: rate of fetal growth that is less than normal in light of the growth potential of that specific infant [7]. The fetus or the new-born is smaller than normal for their gestational age because of reduced growth rate inside the womb.
- Small for gestational age: fetuses or new-borns, smaller in size than normal for their gestational age, with weight below the 10th percentile for the gestational age [8].

In the EMaBS, gestational age was not estimated as ultrasound scanning was not a routine antenatal care procedure. Thus, it is not possible to differentiate between IUGR and SGA.

4.3 The Entebbe Mother and Baby Study

PhD thesis objectives 2, 3, 4 and 5 were addressed using an observational study with prospectively collected longitudinal data in the EMaBS birth cohort from Wakiso district, Uganda.

4.3.1 Design of the Entebbe Mother and Baby Study

The EMaBS comprises of two phases. The first phase was a deworming treatment trial [ISRCTN32849447] in 2,507 healthy pregnant women and their 2,345 live born offspring up to five years of age. This has been followed by an observational follow-up phase in the offspring after five years of age.

The first (trial) phase of the EMaBS began in early 2000s. Between April 2003 and November 2005, healthy pregnant women in their second or third pregnancy trimester were enrolled into a deworming trial. This phase was completed in May 2011 when all offspring had turned five years of age. The trial investigated the impact of helminth infections and anthelminthic treatment in pregnancy and in early childhood on the incidence of infectious and atopic disease events in childhood (including pneumonia, diarrhoea, malaria, measles, tuberculosis, human immunodeficiency virus (HIV), atopic eczema, allergic rhinitis, conjunctivitis, urticaria and wheeze) and vaccine (BCG, measles and tetanus toxoid) immune responses in childhood. It was hypothesised that helminth infections modulate the host immune response both to themselves and to unrelated immunogens, pathogens and allergens. An overview of the EMaBS trial protocol has been published elsewhere [9].

For each child the trial phase ended when they reached five years of age. Overall, the trial ended on 6th May 2011. Results from the trial phase showed that helminths and their treatment in pregnancy had no effect on infants' response to vaccines; maternal gestational hookworm infection was inversely associated with childhood eczema [10]; anthelminthic treatment in pregnancy was associated with increased incidence of infantile [11] and childhood eczema [12]; and albendazole treatment in childhood was associated with reduced childhood malaria [12].

The second (follow-up) phase of the EMaBS began in 2008. For each child, the follow-up phase started when the intervention was completed i.e. when they reached five years of age. The participants continued to be seen annually (as close to their birthday as possible). The follow-up phase aimed at investigating the long-term impact of helminth infections and anthelminthic treatment in pregnancy and early childhood on outcomes such as asthma, eczema and allergies between five and 13 years of age. Consent was obtained to collect additional anthropometric information and examine stool for helminthic infections.

It was hypothesised that early-life (pre-natal and pre-school) exposure to helminth infections provides long-term protection against asthma, eczema and allergies; and that anthelminthic treatment in pregnancy and early childhood increases susceptibility to asthma, eczema and allergies in children.

Results from the follow-up phase showed that i) sensitisation to allergen started early in childhood and increased with age, ii) wheeze and eczema declined as children grew older and were less common by nine years of age [13] , and iii) there was no effect of gestational

treatment with albendazole or praziquantel or of childhood quarterly treatment with albendazole on wheeze in the preceding 12 months or on skin prick testing response at nine years of age [14].

4.3.2 Study setting and population

The EMaBS was conducted in Entebbe Municipality and the neighbouring Katabi sub-county (Figure 4.3-1) in Wakiso district, Uganda; a peninsula on the northern shores of Lake Victoria at 0.05 degrees north and 32.46 degrees east.

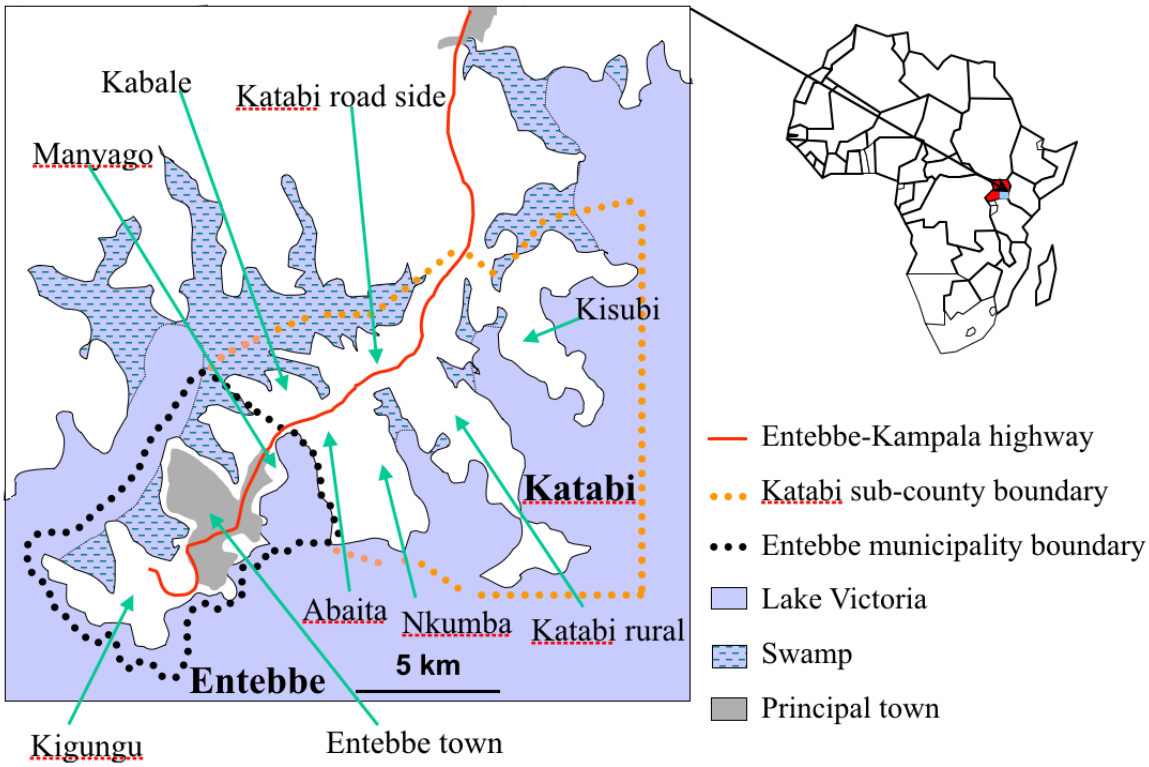

Figure 4.3-1: Map of the Entebbe Mother and Baby Study catchment area[1]

Entebbe is located approximately 40 km southwest of the Ugandan capital, Kampala. The study area is a community in transition undergoing rapid urbanisation, especially along the Kampala-Entebbe highway. Figure 4.3-2 shows the settings of the study area.

The Uganda National Population and Housing Census of 2014 reported that Entebbe municipality and Katabi sub-county had 69,958 individuals and 104,335 individuals respectively [15]. Overall in 2014, Uganda's population was 34.6 million people, an increase of 10.4 million people from the 2002 census, representing a population annual

[1] Source Prof. Alison Elliott (MRC/UVRI & LSHTM Uganda Research Unit)

growth rate of 3% between 2002 and 2014 [15]. In the same period, Wakiso district population grew at a 7% annual rate. In 2014, 46% and 51% of Ugandans were aged 0 to 14 years and 15 to 64 years, respectively [15]. The Baganda at 17% and Banyankole at 10 % are the largest tribes in Uganda [15]. At enrollment, 49% of the women in the EMaBS were of the Baganda tribe [16].

Figure 4.3-2: Showing the setting for the Entebbe Mother and Baby Study catchment area.

Results from the Uganda Demographic and Health Survey (UDHS) 2016 found that the median number of years of formal education reported by adult women had increased from 1 year in 1995 to 3 years in 2016 [17]. In Uganda as a whole, 19% of women have no formal education [17]. At enrollment in the EMaBS, 4% of the women had no education, 50% had primary education, 37% had senior education and 8% had tertiary education. Data from the UDHS further showed that 17% of women (15 to 49 years of age) were employed, of these 50% were involved with agriculture [17]. In Uganda, agriculture is mainly subsistence agriculture. At enrollment in the EMaBS, 63% of the women were

unemployed or housewives and 82% of the women had a monthly personal income of less than 30,000 Ugandan shillings (US$20) [18]. Women enrolled in EMaBS were on average, more highly educated and with a higher income than Ugandan women as a whole.

The EMaBS was based at Entebbe Hospital, the district referral hospital where the majority (over 70%) of pregnant women in the study area attend for antenatal care [19]. The sixth round of the UDHS conducted between June and December 2011, reported a 10% prevalence of low birth weight among Ugandan newborns [20]. In the EMaBS birth cohort, the prevalence of low birth weight (<2.5 kg) was 8% [4].

Prevalence of stunting, wasting, being underweight and being overweight among children aged 6-59 months was 29%, 4%, 11% and 4%, respectively, in the UDHS 2016 report [17]. At one year of age, 14% of the EMaBS participants were stunted, 8% were underweight and 4% were wasted [21]

Helminths and malaria are endemic to the study area. Worm prevalence among pregnant women in Entebbe has declined since the inception of the EMaBS. Between 2003 and 2005, the prevalence of helminths in pregnant women in Entebbe Hospital was 68% [12], but dropped to 26% in 2009 (A. Elliott, unpublished data). Largely, in this birth cohort, the annual worm prevalence in the offspring has been low [12], less than 10% for a given worm type, details shown in Figure 4.3-3.

The Uganda Population-Based HIV Impact Assessment (UPHIA), conducted between August 2016 and March 2017 showed that HIV prevalence was 8% among women aged 15 to 64 years, with a higher burden among urban women at 10% compared to 7% of women living in rural areas. In children (0-14 years of age) HIV prevalence was 1% [22]. The national HIV prevalence was lower than the EMaBS prevalence of 12% in pregnant women [16] and 2% in children [23].

In Entebbe, malaria transmission occurs all year round, with peak transmissions corresponding to the peak rainfall [24]. The area enjoys an equatorial climate with two rainy seasons: the first rains from March to May and the second rains from September to November [24]. Among the EMaBS offspring, malaria incidence in the first five years of life was 34 episodes per 100 child-years with the highest incidence in the first two years of life. The annual prevalence of asymptomatic malaria between the ages 1 and 5 years varied between 5-7% [25].

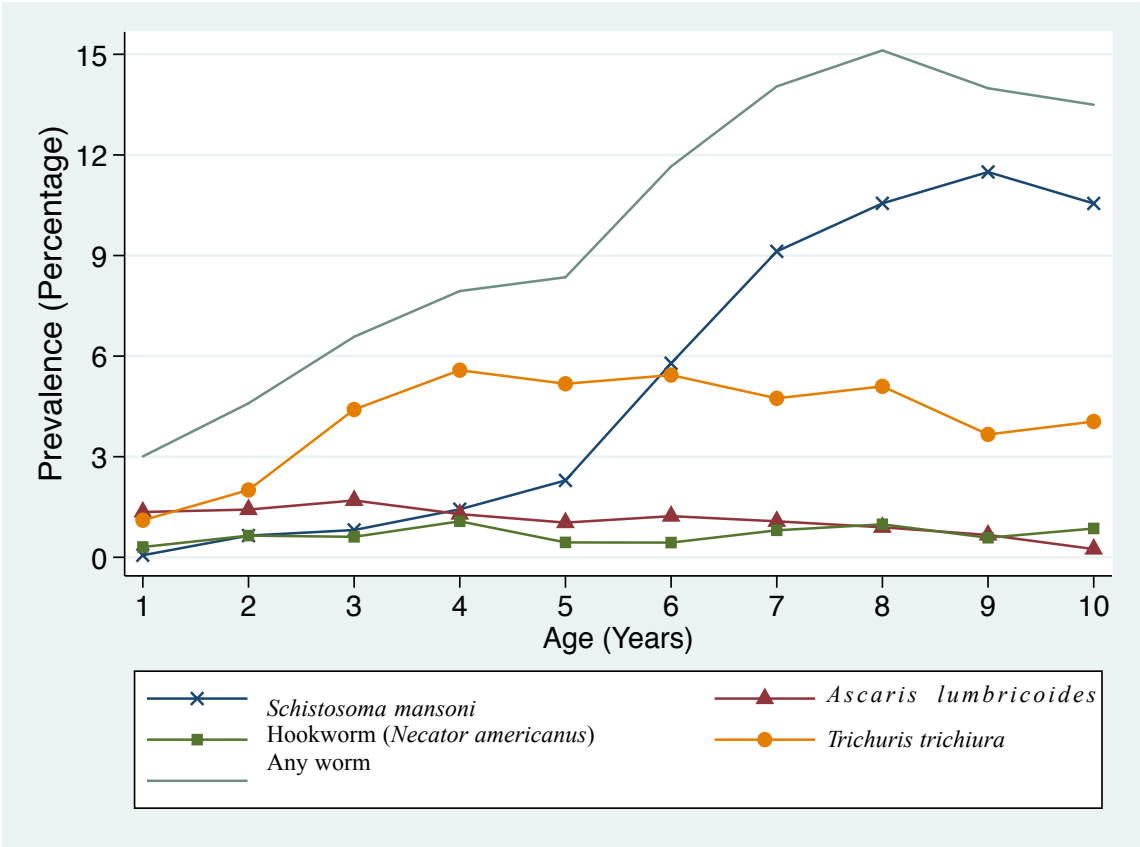

Figure 4.3-3: Worm prevalence by age among the Entebbe Mother and Baby Study birth cohort offspring

As earlier described elsewhere [4, 18], 15,035 women were registered at the antenatal clinic at Entebbe hospital during the recruitment period, of these 11,783 were screened and 3,163 were eligible for enrollment. The most common reasons for ineligibility were residence outside the study area (n=6,243), lack of willingness to have an HIV test (n=1,186), unwillingness to participate in the study (n=874) and enrolment during an earlier pregnancy (n=115) [16, 18]. Of the 3,163 eligible women, 2,507 were enrolled [16, 18], eight were excluded because of double enrolment (as a result of enrolment for an earlier pregnancy) and 596 were lost to follow-up [18]. Those lost to follow-up were screened but then did not come back to the hospital for enrolment. There were no major differences between women enrolled and those screened but not enrolled, except that women screened and not enrolled were more likely to be primigravida or younger [18]. A detailed flow of participants in the EMaBS is provided in Figure 5.2-1, chapter 5.

A community-wide household survey conducted in July to August 2008, surveyed 173 community children aged 3 to 5 years in the study area and compared these children to 199 EMaBS children of the same age who attended the study clinic during the same time period. This study found that 60% of the mothers in the community-based household

survey would have been eligible for enrolment into the trial, of these 30% had been enrolled [26]. Overall, mothers enrolled into the trial had higher educational status and, or better employment status than their community-survey contemporaries. The EMaBS children were more likely to use footwear or sleep under an insecticide treated bed net than the community-based comparison group. However, there were no differences in reported incidences of common infections between community and EMaBS children [26].

4.3.3 Maternal procedures

4.3.3.1 Enrolment

At enrolment, information on women's anthropometry, family, general health and socio-demographic characteristics was collected. Blood and stool samples were collected and examined for infections. Pregnant women were enrolled if they were in their second or third trimester of pregnancy, planning to deliver in hospital, willing to know their HIV status and residing in the study area. They were excluded if they had evidence of helminth-induced pathology (haemoglobin <8 g/dl, severe liver disease or bloody diarrhoea), history of adverse reaction to anthelminthics, abnormal pregnancy (as assessed by the midwife) or were enrolled for an earlier pregnancy [19]. Those with evidence of helminth-induced pathology were investigated, treated with albendazole and haematinics, and transfused with blood if necessary.

As part of routine antenatal care, pregnant women received care comprising of: history taking, clinical examination, anthropometric measurements, daily haematinics (ferrous sulphate and folic acid), intermittent presumptive treatment (sulfadoxine/pyrimethamine) for malaria at 16 and 26 weeks of gestation, tetanus immunisation and treatment for common infections including sexually transmitted infections, and HIV counselling and testing was also done.

4.3.3.2 Randomisations and trial intervention

Participants were randomised to three interventions at two time-points, in a (2x2)x2 factorial design; 2x2 in pregnant women and x2 in their resulting offspring, as shown in Figure 4.3-4. Randomisation codes were generated by the trial statistician using Stata version 7 (College Station, Texas, USA), with numbers allocated in blocks of 100 and 80 for the mothers' and children's treatment groups, respectively; all three randomisations were independent of each other. Colleagues at the MRC/UVRI & LSHTM Uganda Research Unit in Entebbe who were otherwise not working on the trial packed and sealed the interventional drugs in envelopes labelled with allocation numbers.

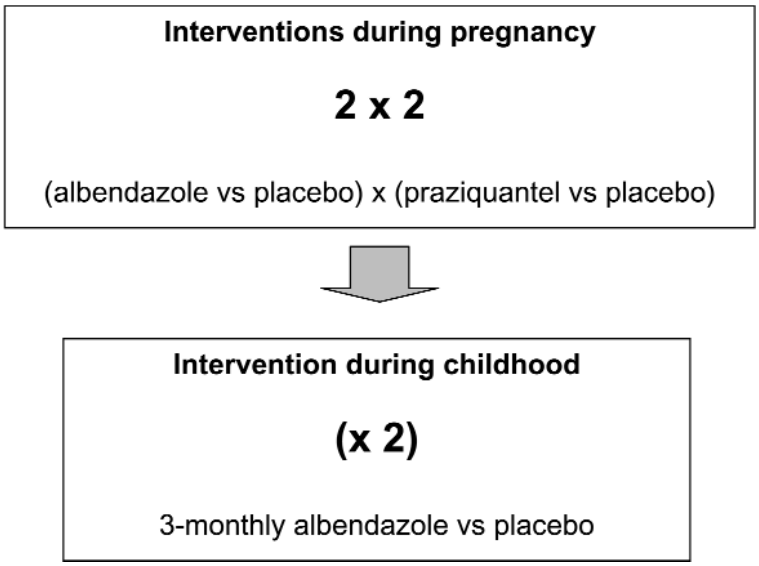

Figure 4.3-4: The three randomisations at two time points[2]

Intervention treatments were allocated to women at enrolment in a numerical order by trained interviewer-counsellors; who also administrated the interventions. Women received single-dose oral albendazole or matching placebo (400mg; GlaxoSmithKline, Brentford, UK) and single-dose oral praziquantel or matching placebo (40mg/kg; Medochemie Ltd, Limassol, Cyprus). All women received albendazole and praziquantel, six weeks after giving birth.

4.3.3.3 Infections and illnesses

Stool samples collected at enrolment were examined for helminths (hookworm [Necator americanus], *Schistosoma mansoni*, *Trichuris trichiura* and *Ascaris lumbricoides*) using Kato-Katz methods [27] and for *Strongyloides stercoralis* using charcoal culture [19]. Blood was examined for microfilariae (*Mansonella perstans*) and malaria parasites using modified Knott's method and Leishman's stain, respectively [28]. Thick blood smears were examined for *Plasmodium falciparum* ring forms or gametocytes by reading fields through 200 white blood cells and at least 100 high-power fields were examined before a film was declared negative.

HIV serology was performed on whole blood using the serial triple rapid testing algorithm with: Determine, (Abbott Laboratories Co. Ltd., Tokyo, Japan); Statpack (Chembio Diagnostic Systems, Medford, NY, USA); and Unigold, (Trinity Biotech plc, Bray,

[2] Source Prof. Alison Elliott (MRC/UVRI & LSHTM Uganda Research Unit)

Ireland) [19, 21]. Only samples positive on Determine were re-tested on Statpack. Discordant samples on Determine and Statpack were further tested using Unigold. Women were considered HIV positive if they were positive on at least two test kits and HIV negative if they were negative on Determine or both Statpack and Unigold.

HIV positive women were offered single-dose oral nevirapine (200 mg) and single dose-oral neonatal nevirapine (2 mg/kg) for use at the onset of labour and within one week of delivery, respectively for prevention of mother-to-child HIV transmission. HIV positive women were also offered daily cotrimoxazole (sulfamethoxazole/trimethoprim) 960 mg for prophylaxis against opportunistic infections. They were referred for antiretroviral therapy (ART) if their CD4 cell counts were ≤200 cells/µl or if they had WHO clinical stages 3 or 4 HIV infection, irrespective of their CD4 count.

4.3.4 Childhood procedures

4.3.4.1 Growth assessment

In Entebbe hospital, birth weight was measured immediately after birth using baby scales (Fazzini SRL, Vimodrone, Italy) to the nearest 0.1 kg, while for those delivered elsewhere, birth weight was recorded as it appeared on the child health card. Offspring in the EMaBS were seen regularly at the study clinic. At six weeks and six months of age, children were weighed using CMS weighing equipment, model MP25 [Chasmors Ltd, London, UK]). Thereafter they were weighed annually starting at one year of age using Seca weighing scales (Seca: gmbh & co.kg, Hamburg, Germany).

Height was measured to the nearest 0.1 cm as recumbent length at six weeks and six months of age using an adjustable child-length measuring board (Seca; Vogel & Halke, Hamburg, Germany). From one year of age, height was measured annually (as close to the child's birthday as possible) using stadiometers (Seca 213; gmbh & co.kg, Hamburg Germany).

4.3.4.2 Trial intervention

At 15 months of age, children were randomized to receive quarterly oral albendazole or matching placebo until the age of five years. Trained study nurses allocated the randomisation codes sequentially and administered the intervention drug. They received syrups (5ml) equivalent to 200mg albendazole or matching placebo labelled according to their randomisation code up to 21 months of age. From 2 to 5 years of age, they received chewable albendazole (400mg) tablets or matching placebo, packaged and sealed in

envelopes labelled according to the randomisation code. Study participants and staff continue to be blinded to the drug allocation both in pregnancy and in childhood.

4.3.4.3 Follow-up, infections and illnesses

Offspring were seen regularly at the study clinic for scheduled visits or when ill. Through home visits and telephone calls, clinic-based fieldworkers reminded participants of their appointments and invited them for new sub-studies or additional procedures.

During the scheduled visits (at six weeks, six months of age and then annually [as close to the child's birthday as possible], starting at one year of age) additional data was collected from the offspring. Stool samples were examined for helminths using Kato-Katz methods [27], while blood samples were examined for malaria parasites using Field's stains [A and B] and for *Mansonella perstans* using modified Knott's method [28].

Children with clinical malaria, asymptomatic malaria or worm infections were treated accordingly. Initially, asymptomatic or simple malaria was treated with chloroquine (25mg base/kg) for three days then combined therapy of chloroquine (25mg base/kg) for three days with single-dose Fansidar (sulfadoxine and pyrimethamine) was used. Currently, simple malaria is treated with oral artemisinin-based combination therapies (artemether and lumefetrine). Complicated malaria is managed with parenteral intravenous quinine 10mg/kg every eight hours in 10-20 ml/kg of 5% dextrose initially until child is able to tolerate oral therapy. This was followed with either oral quinine at 10 mg/kg every 8 hrs until completion of a 7-day course or a full treatment course of an oral artemisinin-based therapy.

Schistosomiasis was treated with praziquantel (40mg/kg). Single-dose albendazole (200mg if <2 years and 400mg for those ≥2years) was used to treat *Ascaris*, hookworm and *Trichostronglyus. Strongyloides* and *Trichuris* were treated with albendazole (200mg if <2 years and 400mg if ≥2years) twice daily for 3 days.

Sick participants were seen at the study clinic, where treatment was provided free of charge. They were managed following the national treatment guidelines at the time. Those requiring hospitalisation were admitted to Entebbe hospital and followed until they were discharged.

At six weeks of age, dried blood spots from children exposed to maternal HIV in pregnancy were investigated for vertical HIV transmission using polymerase chain reaction (PCR) and quantitative reverse transcription (RT)-PCR. After 18 months of age, HIV status of children exposed to maternal HIV in pregnancy was determined using the

serial triple rapid testing algorithm starting with Determine, (Abbott Laboratories Co. Ltd., Tokyo, Japan), followed by Statpack, (Chembio Diagnostic Systems, Medford, NY, USA) and then Unigold, (Trinity Biotech plc, Bray, Ireland) [19, 21]. Children were considered HIV positive if they had a positive PCR test or were positive on the rapid testing algorithm and HIV negative if they were negative on both PCR and the serological serial testing algorithm.

Infants born to HIV positive women received daily prophylaxis with cotrimoxazole (sulfamethoxazole/trimethoprim, 40mg/200mg per 5ml). Cotrimoxazole was discontinued if they were HIV negative after 18 months of age. HIV positive children were started on ART if their CD4 percentage was ≤15% or had WHO clinical stage 3 or 4 HIV infection(s).

4.3.4.4 Whole-genome genotyping

In 2012, ethical approval was granted to generate genotypic data from stored (at -80ºC until processing) samples collected at one year of age. Whole-genome genotyping of 1,391 infants was done, aimed at identifying and characterising mechanisms of human genetic variants impacting on immune responses and protective efficacy of vaccines delivered in early infancy. DNA was extracted from the erythrocyte layer using Qiagen QIAamp DNA Mini or Midi Kits (Qiagen, Hilden, Germany) following recommended protocols.

Approximately 2.2 million genetic variants were generated at Wellcome Trust Sanger Institute in collaboration with the University of Oxford, using the Illumina HumanOmni2.5M-8 ('octo') Beadchip arrays, version 1.1 (Illumina Inc., San Diego, USA). Whole genome was amplified and fragmented before hybridisation of genomic DNA to specific oligonucleotides bound to 3µm diameter silica beads. Genotypes were called from intensities using two clustering algorithms (Illuminus and GenCall) in GenomeStudio (Illumina Inc., San Diego, USA) incorporating data from proprietary pre-determined genotypes.

Quality control (using standard pipelines) was done at the Wellcome Centre for Human Genetics, University of Oxford, to remove individuals and variants with high levels of missingness or deviations from expected levels of heterozygosity or Hardy-Weinberg equilibrium (p-value $<1x10^{-8}$) using standard PLINK (version 1.7) commands (run on Unix) [29].

4.4 Blood Pressure Study

Extra ethical approvals were granted to study BP in 10- and 11-year olds participants in the EMaBS. Between 20th May 2014 and 16th June 2016, information on BP was collected from adolescents attending the study clinic for their annual visit.

4.4.1 Inclusion and exclusion criteria

Adolescents were included in the BP study if they were aged 10 or 11 years and provided additional consent and assent. They were excluded if they were aged 11 years and had previously enrolled in the BP study at 10 years of age. Enrolment was postponed for those with malaria (fever with malaria parasites) or other illness until they were well after being treated by the study team.

4.4.2 Adolescence procedures

Supplementary clinical procedures were done and additional questionnaires on participants' medical and family history, diet and physical activity were completed in the presence of both the participant and their parent or guardian.

4.4.2.1 Anthropometry

Additional anthropometric information on weight, height and waist circumference at the time of BP measurement were collected using Seca weighing scales, Seca stadiometers and Seca tape measures (Seca 201; Seca: gmbh & co.kg, Hamburg, Germany) respectively. Waist circumference was measured using a tape measure at the mid-way position between the lowest rib and the iliac crest and recorded to the nearest 0.1 cm.

4.4.2.2 Blood pressure assessment

After five minutes rest, trained nurses measured BP thrice five minutes apart on the right arm supported at the heart level with the participants seated upright all the way to the back of the chair with their legs uncrossed and feet flat on the floor. Omron (M6) machines fitted with appropriate-sized cuffs were used for BP measurement [3]. Figure 4.4-1 shows a study participant having her BP measurements taken.

For purposes of clinical assessment and care, the mean of the three readings was estimated. The Centers for Disease Control and Prevention (CDC) height percentile charts and the 2004 updated National Health and Nutrition Examination Survey (NHANES) Working Group on Children and Adolescents BP tables were used to obtain BP percentiles [1, 3]. Those with systolic and or diastolic BP ≥95th percentile for sex, age and height on this first occasion (day-one) were invited for repeat BP measurements on up to

two additional occasions one to three weeks apart. Adolescents with BP (systolic and or diastolic) <95th percentile for sex, age and height percentile on the second day were not invited for repeat BP measurement for a third occasion. Those with sustained BP (systolic and or diastolic) ≥95th percentile for gender, age and height on the third day were further investigated and referred for specialist evaluation at Mulago National Hospital.

Figure 4.4-1: Entebbe Mother and Baby Study participant undergoing blood pressure measurement at 10/11 years of age.

Non-pharmacologic measures (emphasising a healthy diet rich in fruits and vegetables; reducing dietary sodium intake; maintaining normal body weight; and regular physical exercise such as runs and walks) were recommended to participants with BP (systolic and or diastolic) ≥90th percentile for sex, age and height.

4.4.2.3 Puberty and the Tanner stages

From 6th January 2015 to 16th June 2016, trained clinicians assessed puberty based on breast and pubic hair development adolescents enrolled in the BP study following Tanner's method [30, 31].

4.4.2.4 Body composition assessment

Extra ethical approval was obtained to collect body composition data from 10- and 11-year old participants in the EMaBS blood pressure study. Between 21st January 2015 and

23rd December 2015, body composition parameters including fat mass (FM, the total weight of fat mass in the body), fat free mass (FFM, the total amount of muscle, bone, tissue, water and all other fat free mass in the body) and total body water mass (TBW, the amount of water retained in the body) were collected using a segmental body composition analyser machine (TANITA BC-418, TANITA Corporation, Tokyo Japan). Briefly, barefooted adolescents stood upright on the posterior electrode base while simultaneously holding strongly onto the two anterior electrodes handles of the body composition analyser machine.

4.4.3 Quality control

The Vector Control Programme at the Ministry of Health, Uganda, provided quality control for Kato-Katz analyses, while malaria parasitology quality control was provided by the UK National External Quality Assessment Schemes.

The MRC/UVRI & LSHTM Uganda Research Unit laboratories in Entebbe provided HIV quality control testing using the non-rapid enzyme-linked immunosorbent assay (ELISA) tests on a proportion of samples which included all samples with discordant results from the serological serial testing algorithm.

The CMS weight scales were regularly calibrated using a standard ten-kilogram stone, made for this purpose. The Omron (M6) digital BP machines were validated and calibrated every six months by the Uganda National Bureau of Standards.

4.4.4 Sample size and study power

The sample size of 1,119 was determined by the number of adolescents enrolled into the BP study. Of these, 932 (83%) had a birth weight record of whom 65 (7%) had low birth weight (birth weight <2.5kg). Respectively, the SD for systolic and diastolic BP was 8.2 mmHg and 7.3 mmHg. Table 4.4-1 shows the power to detect a difference in mean systolic or diastolic BP between low birth weight and non-low birth weight children at a 5% significance level among the 932 adolescents with a recorded birth weight. Birth weight was also analysed as a continuous explanatory variable thus yielding greater power to detect effects on BP than when using dichotomised birth weight variables [32].

Table 4.4-1:Power calculations for the Blood Pressure Study (N=932)

Increase in BP (mmHg)	Systolic BP	Diastolic BP
4.0	96.6%	98.9%
3.5	91.2%	96.1%
3.0	81.1%	89.1%
2.5	65.9 %	75.8%
2.0	47.9 %	56.7%
1.5	29.5%	35.8%

N; numbers of participants, BP; blood Pressure

The genetic analysis included 815 participants with both genotypic and phenotypic data. Table 4.4-2 shows the detectable difference for 80% or 90% power to detect an association at genome-wide significance level (p-value $<5x10^{-8}$) for a range of minor allele frequencies and assuming SD of 8.2 mmHg and 7.3 mmHg for systolic BP and diastolic BP, respectively.

Table 4.4-2: Power calculations for the Genetic Association Study (N=815)

Systolic BP	Study Power	
MAF	**80%**	**90%**
20%	3.2mmHg	3.4mmHg
15%	3.6mmHg	3.8mmHg
10%	4.3mmHg	4.6mmHg
5%	5.9mmHg	6.3mmHg
1%	13.0mmHg	13.9mmHg
Diastolic BP		
MAF	**80%**	**90%**
20%	2.9mmHg	3.1mmHg
15%	3.2mmHg	3.4mmHg
10%	3.8mmHg	4.1mmHg
5%	5.3mmHg	5.6mmHg
1%	11.6mmHg	12.4mmHg

N; numbers of participants, MAF; minor allele frequency, BP; blood pressure

4.5 Statistical methods

This section describes data management processes, variable/data manipulation and categorisation, and analysis strategies for this PhD work. In addition, succinct descriptions of the statistical methods for each thesis objective are given in each published research paper.

4.5.1 Data management

Clinicians, nurses and laboratory technicians captured data on duplicate pre-coded CRFs which were later checked for consistency, batched and double entered into Microsoft Access (Microsoft Corp., Redmond, WA, USA). The original CRFs have been archived in MRC/UVRI & LSHTM Uganda Research Unit sites at Kyamulibwa and Entebbe with duplicate copies maintained in participants' files at the study clinic.

The PhD candidate generated data cleaning and merging queries which were resolved by the PhD candidate together with the study team by cross-checking with the original and or duplicate CRF copies. Changes were later made in the database, participants' files and archived documents. The process was repeated until no more queries could be generated. Various datasets were merged into a single analysis dataset and unique identifiers dropped from the final analysis dataset.

4.5.2 Categorisation of variables

Data was grouped and re-coded into categories thought to be appropriate for a given variable or analysis. The study area was divided into zones based on topography and settlements using the geographical positioning system (GPS) data. This was grouped into urban (Entebbe town, Abaita and Nkumba trading centres, Kigungu, and roadside villages of Katabi) or rural areas of Kisubi and Katabi extending south to Lake Victoria (Figure 4.3-1) [18]. Household socioeconomic index (1 [lowest] to 5 [highest]) was based on previously done principal components analysis of characteristics that reflected socio-economic status such as household possessions, building materials, and the number of rooms [16]. This grouping for study area and household socioe-conomic status has been used before in previous analysis and publications [16, 18].

Data on monthly rainfall for the study area for the months in which an EMaBS child was born was obtained from the Department of Meteorology in Entebbe. The median of these monthly rainfalls was then calculated and used to categorise season of birth as either dry or wet depending on whether the monthly rainfall was below or above this median value.

Birth weight was considered for analysis both as a continuous variable and a categorical variable, grouped as ≤2.5kg, 2.5 to 2.9kg, 3.0-3.5 kg and >3.5kg or as low birth weight (<2.5kg) verses non-low birth weight (≥2.5kg). Place of delivery was Entebbe hospital or home or others, while feeding status at six weeks was grouped as exclusively breastfed or mixed fed or weaned. Sex of the participants was male or female. Trial interventions

in pregnancy were praziquantel or placebo and albendazole or placebo, whereas in childhood the intervention treatment was albendazole or placebo.

HIV status was either positive or negative for women at enrolment and unexposed, exposed not infected or exposed infected for the offspring. Helminth (*Schistosoma, Ascaris*, Hookworm and *Trichuris*) infections were each coded as infected or not infected. Childhood malaria was investigated as both clinical malaria (history of fever or axillary temperature ≥37.5°C and parasitaemia) and asymptomatic malaria (parasitaemia in absence of fever at any of the annual visits from age one to five years. Family history of hypertension or diabetes was classified as yes or no.

Weight-for-age Z-score (WAZ), weight-for-height-Z score (WHZ), height-for-age-Z score (WAZ) and body-mass-index- for-age Z score (BMIZ) at birth, six weeks of age, six months of age and then annually from one to five years of age was calculated using the 2006 World Health Organization (WHO) standard references [33, 34]. The 2006 WHO references were from a Multicentre Growth Reference Study for the assessment of the growth and development of 0 to 5-year-old children. In addition, BMI at 10/11 years of age was calculated as weight in kilograms divided by height in meters squared. Fat mass index (FMI)=FM (kg)/height(m^2), fat free mass index (FFMI)=FFMI (kg)/height(m^2) and total body water index (TBWI)= TBWI (kg)/height(m^2) were respectively used as indices for FM, FFM and TBW, as scaling by height avoids ambiguities that arise from reporting percentages to tall malnourished persons have similar FM or FFM percentages as shorter well-nourished persons [35].

Puberty was assessed using breast and pubic hair development based on Tanner's method [30, 31] and grouped as pre-pubertal (stage 1) or pubertal (stages 2, 3, 4 or 5). Sleep pattern was based on self-reported duration of night's sleep in hours on a standard night or snoring (yes or no). The type of school attended was day or boarding. Participation in physical education activities at school as yes or no and smoking in the household as yes or no.

Information on diet in the previous month was collected as the numbers of days in a typical week for which a given food (fruits, vegetable, sugared drinks, animal proteins, starchy staple foods) was consumed. This was grouped into 0-2 or 3-7 for fruits, vegetable, animal proteins, starchy staples foods but none, 1-3, or 4-7 for sugared drinks. Grouping for fruits, vegetables sugared drinks, animal proteins and starchy staple food aimed at having roughly similar distributions in the groups. Adding salt to cooked food at the table was yes or no.

4.6 Data analysis plan

4.6.1 Summary

This data analysis plan was developed before analysis was done for each of thesis objectives 2, 3, 4 and 5. The data analysis plan outlines the methods for data handling, processing and analysis for each objective.

Study aim: Investigating the impact of early-life, life-course and genetic factors on blood pressure among young Africans

Inclusion criteria
- Cohort members still under-follow-up
- Age 10 or 11 years
- Consent to additional procedures

Exclusion criteria
- Eleven-year-olds already enrolled at 10 years of age
- Enrolment was postponed for offspring with malaria (fever and parasitaemia) or other illnesses until they were well

Analysis packages
- Stata 14.2
- GCTA version 1.22
- R version 3.4.0

4.6.2 Dealing with missing information

Missing data will be summarised for the main exposures and for the potential confounders. Participants with missing data for a given variable will be omitted from any analysis involving that variable. Except for genetic analysis, missing data will not be imputed but sensitivity analysis will be conducted for birth weight and change in WAZ data at the end of analysis for the relevant objective. For example, missing values for birth weight will be replaced with the maximum birth weight recorded among children with non-missing birth weight, and final analysis models re-run and the sensitivity of results to this simple imputation examined. This process will be repeated with the minimum non-missing birth weight replacing the missing values.

Untyped genetic variants and variants identified for the replication analysis will be imputed at the Wellcome Trust Centre for Human Genomics, University of Oxford using an integrated panel consisting of the 1000 Genomes [36] African genome

variation project (AGVP) [37] and Uganda 2000 Genomes (UG2G); genomes of Ugandan individuals of diverse ethnicity from rural Uganda). SHAPEIT2 (version 2 790) [38] and IMPUTE2 (version 2.3.2) [39] [genotype imputation and haplotype phasing programs] will be used for imputation with settings recommended for African populations.

4.6.3 Testing assumptions underlying linear regression modelling

Graphical methods will be used to assess for linear regression analysis assumptions.

4.6.4 Thesis objective 2: Investigate the relationship between birth weight and BP among Ugandan adolescents

Outcomes	• Mean BP (systolic and diastolic)
Exposures	• Birth weight
Forced variables	• Sex • Age at BP measurement
Confounders	• Maternal factors during antenatal period and, or delivery 1. Socio-demographic 2. Anthropometric 3. Infections and illnesses • Children's characteristics 1. Socio-demographic 2. Anthropometric 3. Infections and illnesses
Sub-group analysis	• Sex • Treatment allocation group • Location (rural or urban) of residence • Season of birth

4.6.4.1 Analysis strategy

Descriptive statistics will be presented as frequencies and percentages for categorical data while continuous data will be presented as either mean and their standard deviation for normally distributed data or median and their interquartile range for non-normally distributed data. Graphical displays will also be used. Early-life characteristics will be compared between offspring who participated and did not

participate in the BP study. Pearson correlation coefficients will be used to examine the relationships between anthropometric variables in childhood and BMI at BP measurement. The means and the 95% confidence interval for the first, second and third BP reading of the first occasion will be assessed and compared.

A modelling approach using linear regression will be fitted separately for systolic BP and diastolic BP. Birth weight will be investigated as both a continuous and categorical exposure variable to understand the pattern of its relationship with the outcome variables and determine if a linear or non-linear relationship best fits the data. Birth weight categories of width 500g (or 250g depending on the distribution of birth weight values) will be generated.

First, the crude association between birth weight and BP (systolic or diastolic) will be determined. Likelihood ratio test will be used to investigate effect modification by gender, season of birth, treatment allocation group and area of residence of the crude relationship between birth weight and BP (systolic or diastolic).

Then models for BP containing birth weight and forced variables as predictor variables will be adjusted for each potential confounder in turn, and factors changing the regression coefficient for the relationship of birth weight on BP by an important amount will be considered for model building. One factor at a time will be added, starting with those having the strongest effect i.e. those, which when adjusted for, cause the largest change in the regression coefficient for the association between birth weight and BP. The process will be repeated for the remaining variables until there are no more variables that change the effect of birth weight on BP by an important amount. Effect modification in the final model will be examined for factors that showed effect modification at crude analysis.

Controlling for many variables may lead to data sparsity and/or multicollinearity (correlation of exposure with the controlled confounders or between two confounders). The change in standard error (s.e) for the effect of birth weight on BP will be used for assessing multicollinearity and where it exists, factors introducing multicollinearity will not be included in the model.

Except for current weight, variables on the causal pathway between birth weight and BP will not be included in the models. Some previous reports have adjusted for current weight (adjusting and not adjusting for current weight remains a controversial topic) thus including current weight in our final model will enable comparison with previous

results. Models with and without adjusting for current weight will be compared and differences summarised.

Finally, sensitivity analysis will be conducted for birth weight. Missing values for birth weight will be replaced with the maximum birth weight recorded among children who are non-missing for birth weight, and final analysis models will be re-run. Results from this simple imputation will be compared to those from the main analysis. This process will be repeated using the minimum non-missing birth weight to replace missing values.

4.6.5 Thesis objective 3: Investigate the relationship between postnatal growth and BP among Ugandan adolescents

Outcomes	• Mean BP (systolic and diastolic)
Exposures	• Postnatal growth (change in weight-for-age Z (WAZ) scores) between birth and 5 years and for each of the shorter growth periods: birth to 0.5 years, 0.5 to 1 year, 1 to 2 years and 2 to 5 years
Confounders	• Factors adjusted for in thesis objective 2 • Breastfeeding status at 6 weeks of age
Sub-group analysis	• Birth weight (categorised into < 2.5 kg and ≥ 2.5 kg) • Sex

4.6.5.1 Analysis strategy

Linear regression modelling will be used to assess the effect of postnatal weight gain between birth and 5 years overall and separately for various growth periods (0 to 6 months, 6 to 12 months, 12 to 24 months and 24 to 60 months) on adolescents' BP. The 2006 World Health Organization (WHO) standard references will be used to estimate the weight-for-age Z (WAZ) scores at birth, six months, 1 year, 2 years and 5 years of age. For each participant, the change in WAZ scores for each of the growth periods defined above will be calculated. Models for the effect of the change in WAZ will be fitted separately for systolic BP and diastolic BP. The crude associations for each postnatal growth period and BP will be determined. To assess the independent effect of each postnatal weight gain period on BP, a multivariable model adjusting for confounders (adjusted for in thesis objective 2) and breastfeeding status at six weeks of age will be run. Each postnatal growth variable will be adjusted for the effect of all

earlier postnatal growth periods. Effect modification by birth weight (as a categorical variable) or sex will be examined using the likelihood ratio (LR) test.

Sensitivity analysis for changes in weight gain will be conducted. The maximum estimated changes in WAZ for those who were non-missing for change in WAZ for a given period will substitute the missing values for changes in WAZ, and the final model analysis will be re-run. Results from this simple imputation will be compared to those from the main analysis. This process will be repeated with the minimum non-missing values of the change in WAZ substituting the missing values.

4.6.6 Thesis objective 4: Identify other factors associated with BP among Ugandan adolescents

Outcomes
- Mean BP (systolic or diastolic)

Exposures
- Maternal factors in pregnancy
    1. Socio-demographic characteristics (age, household socio-economic status, education, marital status and area of residence)
    2. Body mass index
    3. Illnesses and Infections (HIV, malaria, hypertension, worms [microfilaria, *ascaris*, hookworm, *schistosoma* and *Trichuris*])
    4. Trial interventions (praziquantel and albendazole)
- Infancy and early childhood factors
    1. Birth weight
    2. Season of birth
    3. Place of birth
    4. Mode of delivery
    5. Sex
    6. Breastfeeding status at 6 weeks of age
- Late childhood
    1. Infections and illnesses (malaria, HIV and worms)
    2. Trial intervention
- Adolescent factors

	1. Anthropometry (body mass index, waist circumference) 2. Age at BP measurement 3. Physical activity 4. Sleep quality and duration 5. Diet 6. Puberty stage (breast and pubic hair development)
Forced variables	• Sex • Age • Body mass index
Potential confounders	• All the above exposures are potential confounders for other exposures (excluding those on the causal pathway)

4.6.6.1 Analysis strategy

Crude relationships between each factor and the outcome (systolic or diastolic BP) will be fitted separately for systolic and diastolic BP using linear regression methods. The 20% significance level will be used to decide factors to consider for multivariable analysis. This will ensure that potential confounders (which need only be weakly associated with the outcome if they are strongly associated with the exposure, in order to cause confounding) are included.

Multivariable analyses will follow a hierarchical approach following the conceptual framework published in chapter 6. Factors will be added to the model in a sequential process starting with distal factors and the proximal factors last. Factors at the same level will be added at the same time and considered potential confounders for each other and for proximal factors. For each factor, regression coefficients will only be adjusted for those variables at the same level or more distal. Statistical significance will be determined by LR test. A factor crudely associated at p-value <0.2 will still be adjusted for (and the adjusted results shown for it) regardless of its p-value in multivariable models.

Multicollinearity will be assessed for factors thought to be correlated using the change in s.e method and where it exists, the factor introducing multicollinearity will be dropped from the model.

4.6.7 Thesis objective 5: Investigate genetic polymorphisms associated with BP among Ugandan adolescents

Outcomes
- Mean BP (systolic or diastolic)

Main exposure
- Genetic variants (single nucleotide polymorphisms (SNPs))

4.6.7.1 Analysis strategy

Genotypic data from 1,391 EMaBS offspring will be merged with BP data from 1,119 EMaBS participants to generate the analysis dataset of participants with both genotypic and phenotypic data.

The Genome-wide Complex Trait Analysis (GCTA) programme will be used for analysis. Genome-wide association study (GWAS) analyses will be conducted first. Mixed linear model regression analysis including age and BMI as fixed effects will be used. SNPs associated with BP (systolic or diastolic) as a quantitative trait at significance level p-value $<5x10^{-8}$ will be considered as statistically significant. Only SNPs with minor effect allele frequency >0.01 will be reported. If no SNP is identified as associated with BP at this GWAS significance level, then candidate gene analyses will be undertaken. This will involve investigating the strength of the association for these SNPs with BP in the EMaBS participants. Candidate gene analysis will be based on prior knowledge: genetic variants previous reported to be associated with BP (systolic or diastolic) from earlier GWAS at significance level of p-value $<5x10^{-8}$. A Bonferroni corrected p-value threshold will be estimated for statistical significance.

4.7 Ethical considerations and permissions

Parents or guardians of the EMaBS participants provided written, informed consent at the various cohort stages (intervention in pregnancy, intervention in the offspring, genotyping, follow-up phase and BP study). The offspring provided written assent starting from eight years of age to allow for continued follow-up and for participation in the BP study.

The Uganda Virus Research Institute (UVRI) Research and Ethics Committee (REC); the Uganda National Council of Science Technology (UNCST); and the London School of Hygiene & Tropical Medicine (LSHTM) granted ethical approvals for EMaBS, including the subsequent protocol amendments (including genotyping, follow-up and BP study). The Oxford Tropical Research Ethics Committee also granted ethical approval for the

genotyping. Ethical clearance was obtained from the Ethics Committee of the LSHTM to conduct this PhD work, Appendix 1.

The adolescents who sustained a high BP (systolic and/or diastolic $\geq 95^{th}$ percentiles for sex, age and height) after the third occasion were further investigated (additional blood tests were conducted), referred for specialist assessment in Mulago hospital and managed according to appropriate guidelines as mentioned earlier and treatment was offered if required.

Blood pressure assessment carried no major risk to the child except causing very minor discomfort. The process of obtaining blood samples also causes minor discomfort to participants.

Unique identifiers used on study questionnaires to link data to an individual during data cleaning and merging data sets were removed from the final analysis dataset.

4.8 References

1. Falkner, B. and S.R. Daniels, Summary of the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents. Hypertension, 2004. 44(4): p. 387-8.
2. Lurbe, E., et al., 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. J Hypertens, 2016. 34(10): p. 1887-920.
3. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics, 2004. 114(2 Suppl 4th Report): p. 555-76.
4. Ndibazza, J., et al., Effects of deworming during pregnancy on maternal and perinatal outcomes in Entebbe, Uganda: a randomized controlled trial. Clin Infect Dis, 2010. 50(4): p. 531-40.
5. Kramer, M.S., Intrauterine growth and gestational duration determinants. Pediatrics, 1987. 80(4): p. 502-11.
6. Quinn, J.A., et al., Preterm birth: Case definition & guidelines for data collection, analysis, and presentation of immunisation safety data. Vaccine, 2016. 34(49): p. 6047-6056.
7. Sharma, D., S. Shastri, and P. Sharma, Intrauterine Growth Restriction: Antenatal and Postnatal Aspects. Clin Med Insights Pediatr, 2016. 10: p. 67-83.
8. Schlaudecker, E.P., et al., Small for gestational age: Case definition & guidelines for data collection, analysis, and presentation of maternal immunisation safety data. Vaccine, 2017. 35(48 Pt A): p. 6518-6528.
9. Elliott, A.M., et al., The impact of helminths on the response to immunization and on the incidence of infection and disease in childhood in Uganda: design of a randomized, double-blind, placebo-controlled, factorial trial of deworming interventions delivered in pregnancy and early childhood [ISRCTN32849447]. Clin Trials, 2007. 4(1): p. 42-57.
10. Mpairwe, H., et al., Maternal hookworm modifies risk factors for childhood eczema: results from a birth cohort in Uganda. Pediatr Allergy Immunol, 2014. 25(5): p. 481-8.

11. Mpairwe, H., et al., Anthelminthic treatment during pregnancy is associated with increased risk of infantile eczema: randomised-controlled trial results. Pediatr Allergy Immunol, 2011. 22(3): p. 305-12.

12. Ndibazza, J., et al., Impact of anthelminthic treatment in pregnancy and childhood on immunisations, infections and eczema in childhood: a randomised controlled trial. PLoS One, 2012. 7(12): p. e50325.

13. Lule, S.A., et al., Life-course of atopy and allergy-related disease events in tropical sub-Saharan Africa: A birth cohort study. Pediatr Allergy Immunol, 2017. 28(4): p. 377-383.

14. Namara, B., et al., Effects of treating helminths during pregnancy and early childhood on risk of allergy-related outcomes: Follow-up of a randomized controlled trial. Pediatr Allergy Immunol, 2017. 28(8): p. 784-792.

15. National Population and Housing Census 2014-Main Report, U.B.o. Statistics, Editor. 2016, Kampala, Uganda.

16. Muhangi, L., et al., Associations between mild-to-moderate anaemia in pregnancy and helminth, malaria and HIV infection in Entebbe, Uganda. Trans R Soc Trop Med Hyg, 2007. 101(9): p. 899-907.

17. Uganda Bureau of Statistics (UBOS) and ICF, Uganda Demographic and Health Survey 2016. 2018, Uganda and Rockville, Maryland, USA: UBOS and ICF: Kampala.

18. Woodburn, P.W., et al., Risk factors for helminth, malaria, and HIV infection in pregnancy in Entebbe, Uganda. PLoS Negl Trop Dis, 2009. 3(6): p. e473.

19. Webb, E.L., et al., Effect of single-dose anthelmintic treatment during pregnancy on an infant's response to immunisation and on susceptibility to infectious diseases in infancy: a randomised, double-blind, placebo-controlled trial. Lancet, 2011. 377(9759): p. 52-62.

20. He, Z., et al., Prevalence of low birth weight and its association with maternal body weight status in selected countries in Africa: a cross-sectional study. BMJ Open, 2018. 8(8): p. e020410.

21. Muhangi, L., et al., Maternal HIV infection and other factors associated with growth outcomes of HIV-uninfected infants in Entebbe, Uganda. Public Health Nutr, 2013. 16(9): p. 1548-57.

22. Uganda Population-Based HIV Impact Assessment (UPHIA) 2016-2017. 2018.

23. Lule, S.A., et al., Factors associated with tuberculosis infection, and with anti-mycobacterial immune responses, among five year olds BCG-immunised at birth in Entebbe, Uganda. Vaccine, 2015. 33(6): p. 796-804.

24. Odongo-Aginya, E., et al., Relationship between malaria infection intensity and rainfall pattern in Entebbe peninsula, Uganda. Afr Health Sci, 2005. 5(3): p. 238-45.

25. Ndibazza, J., et al., Associations between maternal helminth and malaria infections in pregnancy and clinical malaria in the offspring: a birth cohort in entebbe, Uganda. J Infect Dis, 2013. 208(12): p. 2007-16.

26. Millard, J.D., et al., Assessing the external validity of a randomized controlled trial of anthelminthics in mothers and their children in Entebbe, Uganda. Trials, 2014. 15: p. 310.

27. Katz, N., A. Chaves, and J. Pellegrino, A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo, 1972. 14(6): p. 397-400.

28. Melrose, W.D., et al., An improved Knott's concentration test for the detection of microfilariae. Trans R Soc Trop Med Hyg, 2000. 94(2): p. 176.

29. Purcell, S., et al., PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet, 2007. 81(3): p. 559-75.

30. Marshall, W.A. and J.M. Tanner, Variations in pattern of pubertal changes in girls. Arch Dis Child, 1969. 44(235): p. 291-303.

31. Travers, S.H., et al., Gender and Tanner stage differences in body composition and insulin sensitivity in early pubertal children. J Clin Endocrinol Metab, 1995. 80(1): p. 172-8.

32. Royston, P., D.G. Altman, and W. Sauerbrei, Dichotomizing continuous predictors in multiple regression: a bad idea. Stat Med, 2006. 25(1): p. 127-41.

33. Leroy, L.J., zscore06: Stata command for the calculation of anthropometric z - scores using the 2006 WHO child growth standards. 2011.

34. WHO multicentre growth reference study group, WHO child growth standards based on length/height, weight and age. Acta Paediatr Suppl, 2006. 450: p. 76-85.

35. Mattar, L., et al., Can birth weight predict later body composition in anorexia nervosa? Eur J Clin Nutr, 2012. 66(8): p. 964-7.

36. Genomes Project, C., et al., A global reference for human genetic variation. Nature, 2015. 526(7571): p. 68-74.

37. Gurdasani, D., et al., The African Genome Variation Project shapes medical genetics in Africa. Nature, 2015. 517(7534): p. 327-32.

38. O'Connell, J., et al., A general approach for haplotype phasing across the full spectrum of relatedness. PLoS Genet, 2014. 10(4): p. e1004234.

39. Marchini, J., et al., A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet, 2007. 39(7): p. 906-13.

Chapter 5: The relationship between birth weight and postnatal weight gain, and adolescent blood pressure.

5.1 Introduction

This chapter uses observational data from the EMaBS birth cohort to address thesis objectives 2 and 3, with results presented in research paper 2, "Are birth weight and postnatal weight gain in childhood associated with blood pressure in early adolescence? Results from a Ugandan birth cohort", as published in the International Journal of Epidemiology [268]. In addition, the chapter presents data on the flow of participants in the EMaBS birth cohort, unpublished supplementary tables and results of graphical investigations of the assumptions underlying linear regression analysis, which were not included in the paper for reasons of space.

5.2 Participants' flow in the birth cohort

Briefly, 11,783 women attending antenatal care at Entebbe hospital were screened, 3,163 were eligible for enrollment and 2,507 were enrolled into the EMaBS, resulting in 2,345 live offspring. Of the live born infants, 2,315 (99%) were singleton births. Overall, follow-up in the cohort was higher than anticipated at study design; a 10% loss to follow-up per year was expected [237].

Of the 2,345 live born offspring, 2,218 (95%) were seen at the study clinic at 6 weeks of age and 1,654 (71%) seen at 6 months of age, 1,722 (73%) seen at one year of age, 1,586 (68%) seen at two years of age, 1,510 (64%) seen at three years of age and 1,622 (69%) seen at five years of age.

Respectively, phenotypic data on BP and genotypic data (from stored samples) was available for 1,119 (48%) at 10/11 years of age and 1,391 (59%) of the live born offspring. Both phenotypic and genotypic data were available for 815 (35%) of the live born offspring. Detailed flow of participants in the EMaBS is shown in Figure 5.2-1.

Respectively, detailed characteristics of EMaBS participants with phenotypic data and with genotypic data are presented in supplementary tables 1 (Table S1) in chapter 5 and (Table S1) in chapter 7.

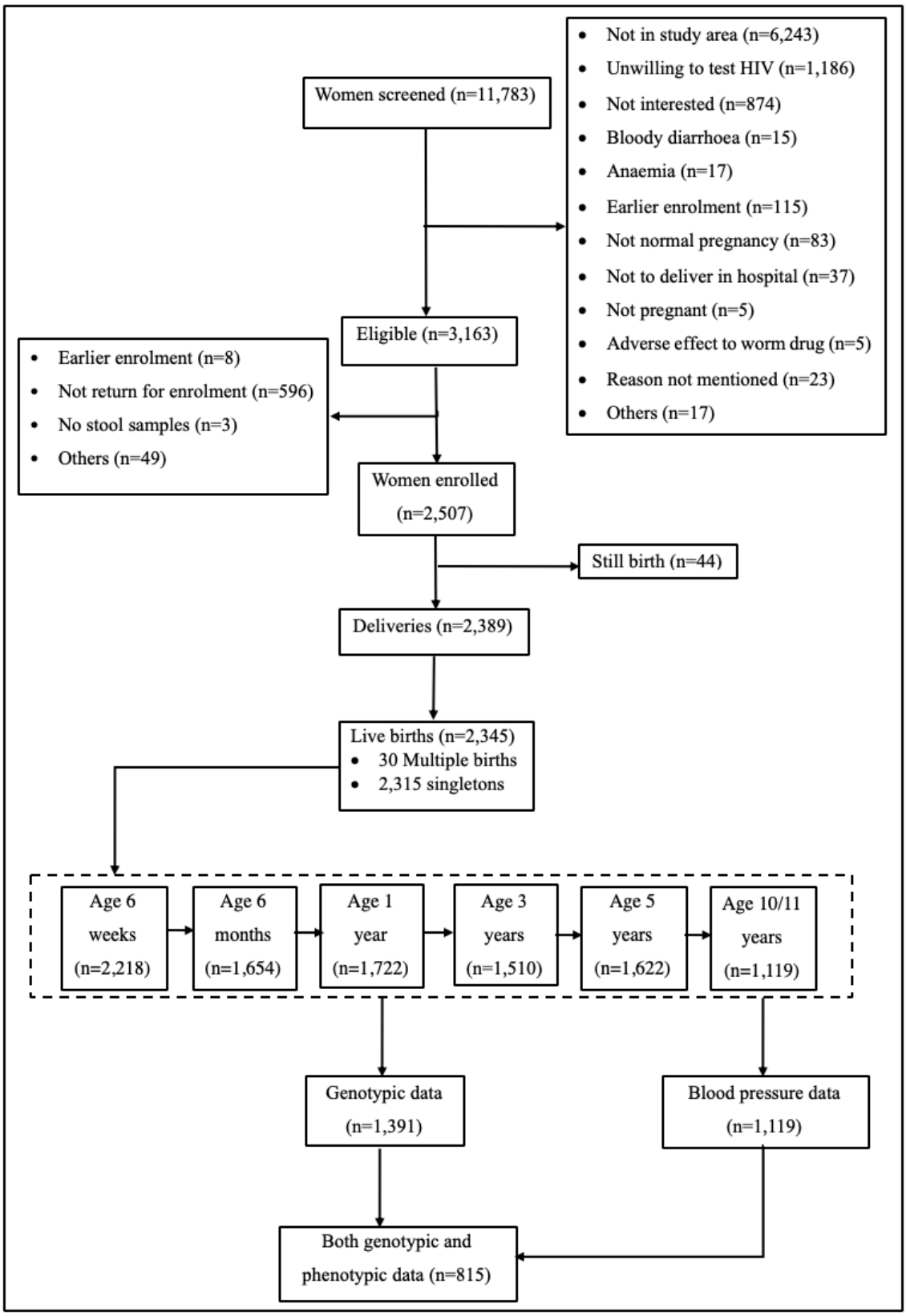

Figure 5.2-1: Showing the number of Entebbe Mother and Baby Study participants seen at selected study time points

5.3 References

1. Lule, S.A., et al., Are birthweight and postnatal weight gain in childhood associated with blood pressure in early adolescence? Results from a Ugandan birth cohort. Int J Epidemiol, 2018.
2. Elliott, A.M., et al., The impact of helminths on the response to immunization and on the incidence of infection and disease in childhood in Uganda: design of a randomized, double-blind, placebo-controlled, factorial trial of deworming interventions delivered in pregnancy and early childhood [ISRCTN32849447]. Clin Trials, 2007. **4**(1): p. 42-57.

5.4 Research paper 2: Are birth weight and postnatal weight gain in childhood associated with blood pressure in early adolescence? Results from a Ugandan birth cohort

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

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SECTION A – Student Details

Student	Abubaker Swaib Lule
Principal Supervisor	Emily Webb
Thesis Title	Investigating the impact of early-life, life-course and genetic factors on blood pressure among young Africans

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

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When was the work published?	03/July/2018		
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	drafted, revised and submitted the final manuscript

Student Signature: _____ **Date:** 02/10/2018

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Original article

Are birthweight and postnatal weight gain in childhood associated with blood pressure in early adolescence? Results from a Ugandan birth cohort

Swaib A Lule,[1,2]* Benigna Namara,[2] Helen Akurut,[2] Lawrence Muhangi,[2] Lawrence Lubyayi,[2] Margaret Nampijja,[2] Florence Akello,[2] Josephine Tumusiime,[3] Judith C Aujo,[4] Gloria Oduru,[2] Liam Smeeth,[5] Alison M Elliott[1,6] and Emily L Webb[1]

[1]Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, UK, [2]Endemic, Neglected, Emerging and Re-emerging Infections Programme, MRC/UVRI Uganda Research Unit, Entebbe, Uganda, [3]Maternity Department, Entebbe Hospital, Entebbe, Uganda, [4]Department of Paediatrics, Mulago Hospital, Kampala, Uganda, [5]Department of Non-communicable Disease Epidemiology and [6]Department of Clinical Research, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK

*Corresponding author. Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, Keppel St, London WC1E 7HT, UK. E-mail: swaiblule@yahoo.com

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Abstract

Background: In Africa, where low birthweight (LBW), malnutrition and high blood pressure (BP) are prevalent, the relationships between birthweight (BW), weight gain and BP later in life remain uncertain. We examined the effects of early life growth on BP among Ugandan adolescents.

Methods: Data were collected prenatally from women and their offspring were followed from birth, with BP measured following standard protocols in early adolescence. Weight-for-age Z-scores (WAZ) were computed using World Health Organization references. Linear regression was used to relate BW, and changes in WAZ between birth and 5 years, to adolescents' BP, adjusting for confounders.

Results: Among 2345 live offspring, BP was measured in 1119 (47.7%) adolescents, with mean systolic BP 105.9 mmHg and mean diastolic BP 65.2 mmHg. There was little evidence of association between BW and systolic [regression coefficient $\beta = 0.14$, 95% confidence interval (CI) (-1.00, 1.27)] or diastolic [$\beta = 0.43$, 95% CI (-0.57, 1.43)] BP. Accelerated weight gain between birth and 5 years was associated with increased BP: systolic $\beta = 1.17$, 95% CI (0.69, 1.66) and diastolic $\beta = 1.03$, 95% CI (0.59, 1.47). Between birth and 6 months of age, effects of accelerated weight gain on adolescent BP were strongest among the LBW (both premature and small-for-gestational-age) children [BW < 2.5 kg: $\beta = 2.64$, 95% CI (0.91, 4.37), BW≥ 2.5 kg: $\beta = 0.58$, 95% CI (0.01, 1.14), interaction *P*-value $= 0.024$].

Conclusions: Findings from this large tropical birth cohort in Uganda suggest that postnatal weight gain rather than BW is important in the developmental programming of BP, with fast-growing LBW children at particular risk. Efforts to control BP should adopt a life course approach.

Key words: Birthweight, postnatal weight change, blood pressure, adolescent, Uganda

Key Messages

- Among Ugandan adolescents, systolic and diastolic blood pressures were positively associated with accelerated weight gain in early childhood, but not with birthweight.
- The effects of accelerated weight gain in the first years of life on later blood pressure were similar among males and females but differed by size at birth, with the strongest effect among those who were born small.
- Blood pressure control strategies should adopt a life course approach rather than focusing specifically on adults or on the prenatal period.

Introduction

Several studies from high-income settings have linked growth in early life (fetal and/or early childhood) with blood pressure (BP) later in life; data suggest an inverse relationship with birthweight (BW) (a proxy for fetal growth)[1] but a positive association with postnatal weight gain (a proxy for growth in childhood).[2] The risk is greatest in individuals who experience a 'nutritional mismatch' (slow uterine growth leading to low BW followed by accelerated postnatal growth).[2] Size at birth, postnatal weight gain and 'nutritional mismatch' could be important in the developmental programming of later BP.

In Africa, where low BW (LBW),[3–6] malnutrition (early life undernutrition and later obesity)[7–9] and high BP[10–12] are prevalent, the relationship between BW or postnatal weight gain and BP later in life remains unclear. Recent systematic reviews showed that mean systolic and diastolic BP varied from 102.0 mmHg to 118.1 mmHg and from 62.0 mmHg to 71.4 mmHg, respectively, in African children and adolescents aged 5–19,[13] whereas the prevalence of high BP varied between 1% and 25% among African children and adolescents aged 2–19 years.[14]

In high-income settings, LBW is mainly due to prematurity, which can be due to any of a number of factors (and their combination), including obstetric conditions, maternal body mass index (BMI), maternal smoking and alcohol intake,[15] whereas in developing countries, small-for-gestational-age (SGA) (often resulting from maternal malnutrition and infections such as HIV and malaria, more prevalent in the tropics) accounts for many LBW infants.[6,16] Other causes of LBW such as maternal alcohol intake and smoking are less common in Africa.

The relationships between BW, postnatal weight gain and BP in low-income settings may differ from those seen in high-income countries. Interventions in pregnancy such as prophylactic antimalarial drugs and anthelminthic treatment, common in these settings, have been shown to be associated with birthweight. For example, maternal treatment with mebendazole in pregnancy was associated with increased birthweight in Nepal.[17] In contrast, our earlier work in Uganda found no impact of maternal treatment with praziquantel and or albendazole in pregnancy on birthweight.[5] However, the impact of such infections and interventions, and of catch-up nutrition in the small or malnourished African infant, on later BP remains unknown and understudied.

Understanding early life determinants of later BP could be vital in the development of interventions that prevent or control high BP before clinical manifestation of subsequent disease(s). We used data from the Entebbe Mother and Baby Study (EMaBS) birth cohort in Uganda, to investigate the relationship between (i) BW and BP in early adolescence, and (ii) accelerated weight gain in childhood and BP in early adolescence.

Methods

Study design and population

Adolescents from the EMaBS, a randomized, double-blind, placebo-controlled trial designed to investigate the effects of worms and their treatment in pregnancy and childhood

on vaccine responses and infections in the children,[18] were enrolled into the BP study.

As previously described,[18] from 2003 to 2005, pregnant women attending antenatal care at Entebbe Hospital and residing in the study area were enrolled and randomized to receive single-dose praziquantel or matching placebo and single-dose albendazole or matching placebo in a 2 x 2 factorial design. Those with evidence of helminth-induced pathology or history of adverse reaction to anthelminthics or abnormal pregnancy, or who had enrolled for an earlier pregnancy, were excluded.[19]

At 15 months, the resulting offspring were randomized to receive quarterly albendazole or matching placebo up to age 5 years.[18] Demographic, socioeconomic and health information was collected prenatally (from pregnant women) and from birth onwards from the live-born offspring.

Measurements

Birthweight was measured immediately after birth using scales (Fazzini SRL, Vimodrone, Italy) for those delivered in Entebbe hospital. For offspring delivered elsewhere, BW was recorded as written on the child health card. Weight was measured at 6 weeks and 6 months of age, using CMS weighing equipment (model MP25: Chasmors Ltd, London, UK) and then annually (close to the child's birthday) using weighing scales (Seca, Hamburg, Germany). Height was measured as recumbent length at age 6 weeks using an adjustable child-length measuring board (Seca, Hamburg, Germany), then annually (from age 1 year) using stadiometers (Seca 213, Hamburg, Germany). BMI was weight in kilograms (kg) divided by height in metres (m) squared.

Children continued under follow-up after the trial intervention ended in 2011. Between 2 May 2014 and 1 June 2016, those attending their visit at ages 10 or 11 years and not presenting with an illness were enrolled in the BP study; 11-year-olds were excluded if they were previously enrolled as 10-year-olds.

Trained nurses measured BP thrice 5 minutes apart, using an appropriate-sized cuff[20] on the right arm supported at the heart level, with the participant seated upright all the way to the back of the chair, legs uncrossed and feet flat on the floor. Automated Omron (M6, HEM-700) machines, validated every 6 months by the Uganda National Bureau of Standards, were used. Means of the three systolic and diastolic BP were calculated. Blood pressure percentiles were obtained using Center for Disease Control height percentile charts and National Health and Nutrition Examination Survey Working Group on Children and Adolescents BP tables.[20,21] Adolescents with mean BP (systolic and/or diastolic) measurements ≥95th percentile for gender, age and height on day 1 had BP measured for up to two extra days. Those sustaining a high BP on day 3 were referred for specialist attention. Non-pharmacological management was recommended to adolescents with BP (systolic and/or diastolic) ≥90th percentile for age, gender and height.

The study was approved by ethics committees of the Uganda Virus Research Institute, the London School of Hygiene and Tropical Medicine and the Uganda National Council for Science and Technology. Written informed consent and assent were obtained.

Statistical methods

Data were double-entered in Microsoft Access and analysed using Stata 14 (College Station, TX, USA). Characteristics of cohort members enrolled and not enrolled in the BP study were compared using chi-square tests. The study outcomes were systolic and diastolic BP. The mean of the second and third day-1 BP measurements was used for analysis, as these were on average different from and lower than the first day-1 measurements (Supplementary Figure 1, available as Supplementary data at *IJE* online).

Key exposures were BW and postnatal weight gain. Postnatal weight gain was change in weight-for-age Z-score (WAZ) between birth and age 5 years, with shorter growth periods (birth to 6 months, 6 to 12 months, 12 to 24 months and 24 to 60 months) also examined. The 2006 World Health Organization standard references[22,23] were used to calculate WAZ, weight-for-height Z-score (WHZ) and BMI-for-age Z-score (BMIZ).

Potential confounders were maternal characteristics including sociodemographic (age, education, area of residence, socioeconomic status), BMI, pregnancy anthelminthic trial interventions, illness and infections (hypertension, HIV, malaria, worms), and child's characteristics including sex, feeding status, BMI, age, childhood anthelminthic trial intervention, illness and infections (malaria, worms).

Pearson correlation coefficients between BMI at age 10–11 years and anthropometric variables (WAZ, WHZ and BMIZ) at birth, 6 weeks, 6 months and annually from 1 to 5 years were calculated. Linear regression (fitted separately for systolic and diastolic BP) was used to assess the association between each key exposure and adolescent BP. Adolescents' age and sex were included a priori in all models. Regression models were adjusted for each potential confounder in turn, with those causing an important change in the effect of the exposure of interest on BP retained in the final model. Final models with and without current weight were fitted.

For BW, we assessed whether a linear or non-linear (categorical or quadratic) relationship provided a better fit to the data. Likelihood ratio test (LRT) was used to

examine for effect modification by gender, original trial interventions and birth season. Since the timing of wet seasons in this setting is subject to variability, birth months were categorized as either dry or wet depending on whether the total monthly rainfall was below or above the median rainfall for all birth months.

For weight gain, as well as for those confounders identified in the BW exposure analysis, the effect of each growth period was adjusted for earlier postnatal weight gain period(s) and feeding status at 6 weeks. Effect modification by gender or BW (<2.5 kg versus ≥2.5 kg) was assessed using LRT.

Sensitivity analysis assessing the impact of missing values for the main exposures was conducted: missing BW values were replaced with the minimum (1.26 kg) and the maximum (5.50 kg) non-missing value of BW recorded, and final models re-run for both scenarios. Similarly, missing values for WAZ change were replaced with the smallest and largest change in WAZ for the given growth period.

Results

Of the 2345 adolescents born into the cohort, 107 (4.6%) had died and 1119 (47.7%) were enrolled. Of those enrolled, 583 (52.1%) were males, 1100 (98.3%) were singletons, 65 (7.0%) had BW<2.5 kg, 824 (73.7%) were delivered and weighed in Entebbe hospital, 651 (58.2%) were delivered in the dry season and 36 (2.9%) were HIV-positive (Supplementary Table 1, available as Supplementary data at *IJE* online).

Participants and non-participants in the BP study were similar for most characteristics (Supplementary Table 1, available as Supplementary data at *IJE* online), with the exceptions that enrolled adolescents were less likely to be HIV positive (*P*-value = 0.049) but more likely to be singletons (*P*-value = 0.048), and born to more educated (*P*-value < 0.001) and married/cohabiting mothers (*P*-value = 0.001), who were less likely to have had hookworm infections (*P*-value = 0.002) at enrolment.

Participants' median age was 10.2 years [interquartile range (IQR): 10.0 to 10.9], mean BW was 3.2 kg [standard deviation (SD) 0.5], mean systolic BP was 105.9 mmHg (SD 8.2; range: 74.5–142 mmHg) and mean diastolic BP was 65.2 mmHg (SD 7.3;range: 44-96.5 mmHg). On day 1, 117 (10.5%) had pre-hypertension (mean systolic and/or diastolic BP ≥90th but <95th percentile for gender, age and height), and 94 (8.4%) had hypertension (mean systolic and/or diastolic BP ≥95th percentile for gender, age and height) of whom 76 (80.9%) had stage one and 18 (19.1%) had stage two hypertension. Of those due for a day-2 BP assessment, 72 (76.6%) returned; of these 23 (31.9%) had pre-hypertension and 24 (33.0%) had hypertension. Finally, 18 (75.0%) of those due for a day-3 BP measurement returned, among whom six (33.3%) had pre-hypertension and seven (38.9%) had hypertension. Allowing for loss to follow-up between days 1 to 3, the estimated hypertension prevalence was 1.1%.

Relationships between anthropometric variables in childhood and BMI at 10–11 years are shown in Figure 1. Correlations between anthropometric variables in childhood and BMI at 10–11 years increased dramatically between birth and 1 year of age, before continuing to increase more gradually. For example, the correlation between WAZ up to and including 6 weeks of age and BMI at age 10–11 years was weak (r < 0.3); the correlation between WAZ at age 0.5–4 years and BMI at age 10–11 years was moderate (0.3≤ r<0.5); and the correlation between WAZ at age 5 years and BMI at age 10–11 years was strong (r≥0.5).

Effect of birthweight on blood pressure

Crudely, there was a slight suggestion of a U- or J-shaped relationship between BW and BP (Figure 2). Compared with those with BW 3.00–3.49 kg, the difference in adjusted mean systolic BP was +0.30 mmHg, 95% CI (−1.87, +2.47), −0.07 mmHg, 95% CI (−1.50, +1.35) and +0.78 mmHg, 95% CI (−0.56, +2.11) among those with BW <2.5 kg, 2.5–2.99 kg and ≥3.5 kg, respectively (Supplementary Table 3, available as Supplementary data at *IJE* online). However, confidence intervals were wide and treating BW as a categorical variable in the regression model did not improve the fit of the model to the data compared with a linear model (*P*-values for departure from

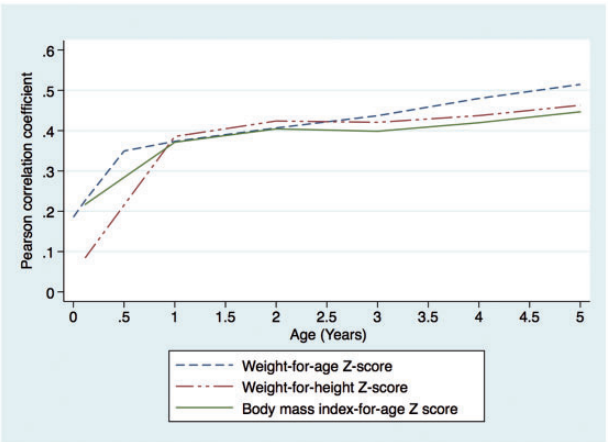

Figure 1. Relationship between anthropometric parameters in childhood and body mass index at the time of blood pressure measurement (10–11 years of age) in adolescents from the Entebbe Mother and Baby Study cohort.

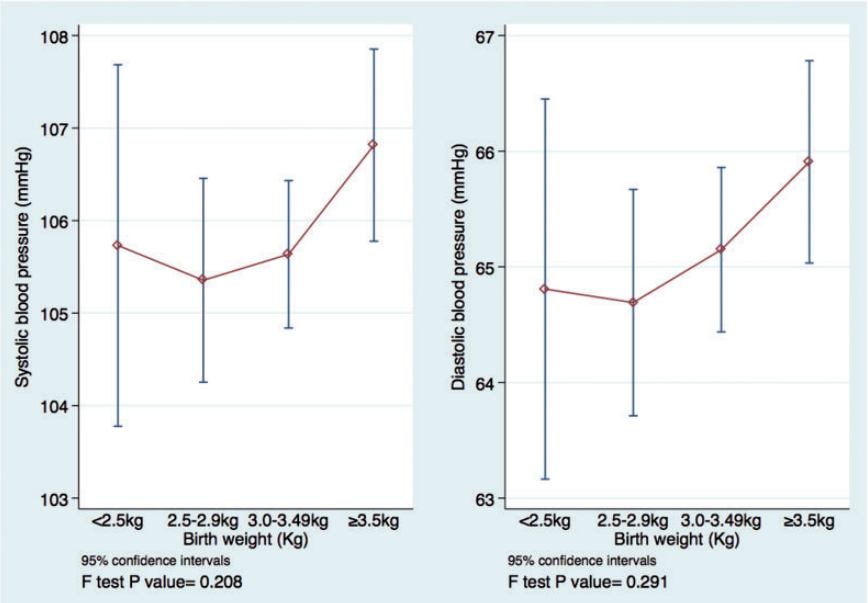

Figure 2. Crude relationship between birthweight and blood pressure among 10- and 11-year olds in the Entebbe Mother and Baby Study.

linearity 0.253 and 0.404 for systolic and diastolic BP, respectively). Regardless of whether or not BW was modelled as categorical or linear, there was no evidence for association between BW and BP; for example, linear model regression coefficients were β = 0.14, 95% CI (-1.00, 1.27) for systolic and β = 0.43, 95% CI (-0.57, 1.43) for diastolic BP (Table 1).

The effect of BW on systolic BP did not differ by sex (interaction *P*-value = 0.464), birth season (interaction *P*-value = 0.515) or maternal deworming drugs (praziquantel interaction *P*-value = 0.230, albendazole interaction *P*-value = 0.594). Likewise, the effect of BW on diastolic BP did not differ by season of birth, child's sex or maternal anthelminthic treatment(s). Additionally, adjusting for current weight, the regression coefficients for the effect of BW on systolic BP were -0.91 mmHg, 95% CI (-1.99, 0.18) and -0.35, 95% CI (-1.32, 0.61) for systolic and diastolic BP, respectively. Results from sensitivity analyses were consistent with the main findings (Supplementary Table 2, available as Supplementary data at *IJE* online); for example, the effect of BW on systolic BP varied from a 0.04-mmHg reduction to a 0.07-mmHg increase in systolic BP (compared with the main analysis β point estimate: 0.14 mmHg).

Effect of weight gain on blood pressure

Crudely, systolic BP was not associated with WAZ at birth [β = 0.34, 95% CI (-0.16, 0.83)] but was strongly associated with WAZ at 6 months [β = 1.14, 95% CI (0.66, 1.61)], 1 year [β = 1.46, 95% CI (1.02, 1.91)] 2 years [β = 1.71, 95% CI (1.23, 2.20)] and 5 years [β = 2.27, 95% CI (1.72, 2.81)]. A similar trend was observed for diastolic BP.

Rapid weight gain between birth and age 5 years was associated with increased BP. Respectively, systolic and diastolic BP increased by 1.17 mmHg and 1.03 mmHg per unit increase in WAZ between birth and 5 years of age (Figure 3). This relationship did not differ by sex or BW (Table 3).

Rapid weight gain during each growth period up to 2 years was independently associated with increased systolic BP, after adjusting for weight gain in the preceding periods. For example, systolic BP increased by 0.77 mmHg, 95% CI (0.26, 1.27) per unit increase in WAZ between birth and age 6 months, by 1.07, 95% CI (0.16, 1.98) between 6 months and 1 year, adjusting for change in WAZ between birth and 6 months, and by 1.21, 95% CI (0.22, 2.20) between 1 and 2 years, adjusting for change in WAZ between birth and 6 months and between 6 months and 1 year (Table 2). Between 2 and 5 years of age, there were no additional effects of increased WAZ on systolic BP among adolescents. For diastolic blood pressure, only rapid weight gain in the first 6 months of life was associated with BP in early adolescence (Table 2).

The effect of accelerated weight gain to age 6 months on systolic BP was stronger among adolescents who were small at birth: β = 2.64, 95% CI (0.91, 4.37) for BW < 2.5 kg and β = 0.58, 95% CI (0.01, 1.14) for BW ≥ 2.5 kg (interaction *P*-value 0.024; Table 3). There was no evidence of effect modification by sex. Results from sensitivity analyses were generally consistent with

Table 1. Crude and adjusted effect of birth weight on blood pressure among Entebbe Mother and Baby Study adolescents

Blood pressure	Number	Mean birth weight (Kg)	Mean BP (mmHg)	Crude association	Adjusted association	
				β (95% CI)	β (95% CI)	β (95% CI)[c]
Systolic BP	1119	3.19	105.87	0.73 (−0.33, 1.80)	0.14 (−1.00, 1.27)[a]	−0.91 (−1.99, 0.18)
Diastolic BP	1119	3.19	65.20	0.66 (−0.27, 1.59)	0.43 (−0.57, 1.43)[b]	−0.35 (−1.32, 0.61)

β; linear regression coefficient: mean difference in blood pressure measured in mmHg per 1 kg increase in birth weight, CI: confidence interval.

[a]Adjusted for maternal factors at enrolment (age, household socioeconomic status, body mass index, asymptomatic malaria, education, parity) and child factors (sex, age, family history of blood pressure).

[b]Adjusted for maternal factors at enrolment (age, household socioeconomic status, body mass index, asymptomatic malaria, education, parity) and child factors (sex, age, asymptomatic malaria, family history of blood pressure).

[c]Additionally adjusted for current weight.

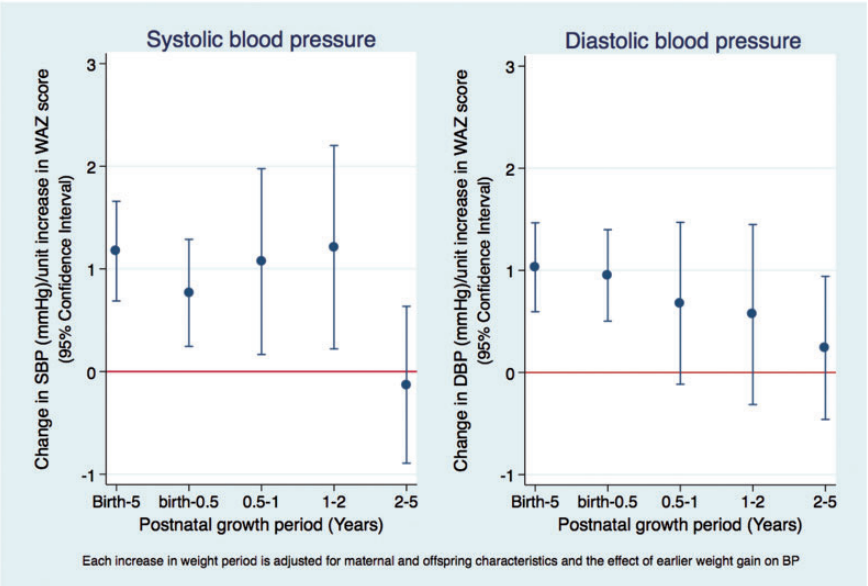

Figure 3. The effect of postnatal weight gain on adolescents' blood pressure in the Entebbe Mother and Baby Study.

those from the main analysis (Supplementary Table 4, available as Supplementary data at *IJE* online).

Discussion

For the first time in mainland East Africa, we described the relationship between BW, postnatal weight gain and BP in adolescents. Birthweight was not associated with BP among early adolescents, but accelerated postnatal weight gain, particularly in the first 2 years of life, was associated with systolic BP, with strongest effects among those born small.

Studies among children and adults have often reported inverse associations between BW and BP, whereas those among adolescents have been inconsistent.[24,25] We found no evidence of association, although the relationship between BW and later BP became more inverse on adjusting for current weight, according with previous studies.[26,27] We reported results adjusted for current weight, to enable comparison with earlier studies that have done so, although current weight could be considered a mediator rather than a confounder.

Studies from Africa have mainly shown no associations between BW and BP among adolescents (reviewed in Lule *et al.*[13]) Exceptions to this were inverse associations observed among adolescents from Kinshasa, and among boys but not girls from Soweto.[28,29] In our study child's sex, worm treatment in pregnancy, or season of birth did not modify the effect of BW on later BP. As hypothesized, accelerated postnatal weight gain was associated with high BP, with the strongest effects among those born small. This suggests that LBW babies may well be at increased risk of later high BP in this setting, not because of being LBW, but because they are more likely to have rapid early weight gain.

Table 2. The association between postnatal weight gain and blood pressure among 10- and 11-year-old adolescents in the Entebbe Mother and Baby Study

Postnatal growth	Number	Crude β (95% CI)	Adjusted β (95% CI)
Systolic blood pressure			
Δ WAZ birth-0.5 year	705	0.71 (0.21, 1.21)	0.77 (0.26, 1.27)[a]
Δ WAZ 0.5-1 year	733	0.76 (−0.04, 1.56)	1.07 (0.16, 1.98)[a]
Δ WAZ 1-2 years	793	0.06 (−0.69, 0.82)	1.21 (0.22, 2.20)[a]
Δ WAZ 2-5 years	802	0.20 (−0.54, 0.94)	−0.13 (−0.89, 0.64)[a]
Δ WAZ Birth-5 years	829	1.17 (0.70, 1.64)	1.17 (0.69, 1.66)[a]
Diastolic blood pressure			
Δ WAZ birth-0.5 year	705	0.88 (0.45, 1.31)	0.95 (0.50, 1.40)[b]
Δ WAZ 0.5-1 year	733	0.45 (−0.25, 1.15)	0.68 (−0.12, 1.47)[b]
Δ WAZ 1-2 years	793	−0.39 (-1.06, 0.27)	0.57 (−0.32, 1.45)[b]
Δ WAZ 2-5 years	802	0.48 (−0.18, 1.15)	0.24 (−0.46, 0.94)[b]
Δ WAZ birth-5 years	829	1.01 (0.59, 1.42)	1.03 (0.59, 1.47)[b]

Δ WAZ: change in weight-for-age-Z score. β: linear regression coefficient: mean difference in blood pressure measured in mmHg per 1 unit increase in ΔWAZ in the specified time interval.

[a]Adjusted for any earlier postnatal growth period(s), maternal factors at enrolment (age, household socioeconomic status, body mass index, asymptomatic malaria, education, parity) and child factors (sex, age, feeding status at 6 weeks, family history of blood pressure).

[b]Adjusted for the earlier postnatal growth period, maternal factors at enrolment (age, household socioeconomic status, body mass index, asymptomatic malaria, education, parity) and child factors (sex, age, feeding status at 6 weeks, asymptomatic malaria, family history of blood pressure).

Table 3. Effect of postnatal weight gain on blood pressure stratified by birth size and sex among adolescents in the Entebbe Mother and Baby Study

Postnatal growth period	**Birthweight <2.5 kg (*n* = 65)**	**Birthweight ≥2.5 kg (*n* = 867)**	**Interaction *P*-value**
	Adj. β (95% CI)	**Adj. β (95% CI)**	
Systolic blood pressure[a]			
Δ WAZ birth-0.5 year	2.64 (0.91, 4.37)	0.58 (0.01, 1.14)	0.024
Δ WAZ 0.5-1 year	−0.13 (−3.44, 3.19)	1.18 (0.23, 2.13)	0.448
Δ WAZ 1-2 years	2.20 (−0.80, 4.20)	1.16 (0.10, 2.20)	0.504
Δ WAZ 2-5 years	−1.00 (−4.182.18)	0.26 (−0.61, 1.13)	0.444
Δ WAZ birth-5 years	2.41 (0.52, 4.30)	1.30 (0.74, 1.87)	0.268
Diastolic blood pressure[b]			
Δ WAZ birth-0.5 year	1.99 (0.49, 3.48)	0.96 (0.47, 1.46)	0.139
Δ WAZ 0.5-1 year	−0.25 (−3.14, 2.65)	0.85 (0.02, 1.68)	0.168
Δ WAZ 1-2 years	0.05 (−2.63, 2.72)	0.82 (−0.12, 1.76)	0.121
Δ WAZ 2-5 years	0.79 (−2.08, 3.66)	0.40 (−0.39, 1.18)	0.793
Δ WAZ birth-5 years	2.08 (0.38, 3.77)	1.20 (0.69, 1.71)	0.323
	Males (*n* = 583)	**Females (*n* = 536)**	***P*-value**
	Adj. β (95% CI)	**Adj. β (95% CI)**	
Systolic blood pressure[a]			
Δ WAZ birth-0.5 year	1.19 (0.50, 1.89)	0.27 (−0.47, 1.01)	0.069
Δ WAZ 0.5-1 year	0.47 (−0.80, 1.74)	1.65 (0.41, 2.89)	0.177
Δ WAZ 1-2 years	1.02 (−0.23, 2.28)	1.47 (−0.02, 2.92)	0.626
Δ WAZ 2-5 years	−0.03 (−1.19, 1.13)	0.40 (−0.81, 1.62)	0.612
Δ WAZ birth-5 years	1.46 (0.80, 2.13)	1.30 (0.51, 2.10)	0.743
Diastolic blood pressure[b]			
Δ WAZ birth-0.5 year	1.27 (0.67, 1.88)	0.57 (−0.08, 1.23)	0.116
Δ WAZ 0.5-1 year	0.41 (−0.70, 1.51)	0.95 (−0.15, 2.04)	0.479
Δ WAZ 1-2 years	0.83 (−0.28, 1.95)	0.20 (−1.09, 1.49)	0.437
Δ WAZ 2-5 years	−0.03 (−1.01, 0.94)	0.54 (−0.48, 1.55)	0.424
Δ WAZ birth-5 years	1.103 (0.59, 1.47)	0.83 (0.16, 1.50)	0.438

Δ WAZ: change in weight-for-age-Z score. β: linear regression coefficient: mean difference in blood pressure measured in mmHg per 1 unit increase in ΔWAZ in the specified time interval.

[a]Each growth period was adjusted for the earlier postnatal growth period, maternal factors at enrolment (age, household socioeconomic status, body mass index, asymptomatic malaria, education, parity) and child's factors (sex, age, feeding status at 6 weeks, family history of blood pressure).

[b]Each growth period was adjusted for the earlier postnatal growth period, maternal factors at enrolment (age, household socioeconomic status, body mass index, asymptomatic malaria, education, parity) and child's factors (sex, age, feeding status at 6 weeks, asymptomatic malaria, family history of blood pressure).

Consistent with literature from high-income countries,[2,25,30] there was a positive association between rapid weight gain and adolescent BP. Few studies from low-middle-income countries have examined the impact of rapid weight gain on later BP. Notably in Seychelles, rapid weight gain was associated with BP in children and adolescents although, contrary to our findings, later rapid weight gain was more strongly associated with later BP than was rapid weight gain in infancy.[31] Our observation that individuals who were small in early life and experienced accelerated postnatal weight had higher levels of BP accords with findings among Senegalese adults[32] but differs from results in Chinese children aged 3–6 years.[33]

Findings relating to diastolic BP were consistent with those for systolic BP; thus fetal growth and postnatal growth have similar influences on both systolic and diastolic BP, except that accelerated weight gain up to 2 years of age was associated with systolic BP whereas the effect of rapid weight gain on diastolic BP occurred during the first 6 months.

Strengths of this study included the availability of well-documented prospectively collected data on important covariates including BW, minimizing recall and reporter bias. Exposures and confounders were determined before the BP study was conceptualized and designed. Robust methods were used to measure BP.

Potential limitations include residual confounding from unmeasured confounders, and selection bias resulting from exclusion of pregnancies considered to be abnormal by the midwife (most likely to result in LBW), thus potentially resulting in an underestimation of LBW prevalence or its impact on later BP. Data on prematurity and SGA were not collected, and therefore we were unable to determine the independent effect of each on later BP. Although 187 (16.7%) adolescents were missing BW data, results from sensitivity analyses were similar to the main analysis findings.

Whereas 52% of the adolescents born into this cohort were not enrolled in the BP study, cohort loss to follow-up was below 60%, a level considered to introduce bias if data were missing completely at random or missing at random.[34] After excluding those who had died by the time of this study, the proportion included in the study was 50%, higher than anticipated (10% loss to follow-up per year was expected).[18] Furthermore, there were no major differences between cohort members enrolled and not enrolled in the BP study. Our findings are generalizable to this cohort but may not be generalizable to the wider population. Our previous work has suggested that cohort members may, on average, be of somewhat higher socioeconomic status than community residents[35] and, as a consequence of study participation, they have received free medical care so their health outcomes may be better than in the general population.

Strategies in childhood that prevent excessive weight gain, especially among infants born small, could be important in preventing later high BP. Disease prevention strategies should enhance optimal growth through good maternal and infant nutrition to reduce both LBW and rapid weight gain in early childhood. Future research should seek to evaluate the impact of catch-up nutritional programmes in malnourished infants or infants born small on BP later in life.

In summary, 'nutritional mismatch' in early life is critical in the developmental programming of BP among adolescents in developing countries. Strategies that prevent LBW and excessive weight gain in early childhood or reverse their effects on BP could prevent high BP and diseases attributable to high BP.

Supplementary Data

Supplementary data are available at *IJE* online.

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References

1. Barker DJ, Bull AR, Osmond C, Simmonds SJ. Fetal and placental size and risk of hypertension in adult life. *BMJ* 1990;**301**: 259–62.
2. Belfort MB, Rifas-Shiman SL, Rich-Edwards J, Kleinman KP, Gillman MW. Size at birth, infant growth, and blood pressure at three years of age. *J Pediatr* 2007;**151**:670–74.

3. Assefa N, Berhane Y, Worku A. Wealth status, mid upper arm circumference (MUAC) and antenatal care (ANC) are determinants for low birthweight in Kersa, Ethiopia. *PLoS One* 2012;**7**: e39957.
4. Denoeud L, Fievet N, Aubouy A *et al*. Is chloroquine chemoprophylaxis still effective to prevent low birthweight? Results of a study in Benin. *Malar J* 2007;**6**:27.
5. Ndibazza J, Muhangi L, Akishule D *et al*. Effects of deworming during pregnancy on maternal and perinatal outcomes in Entebbe, Uganda: a randomized controlled trial. *Clin Infect Dis* 2010;**50**:531–40.
6. Muchemi OM, Echoka E, Makokha A. Factors associated with low birthweight among neonates born at Olkalou District Hospital, Central Region, Kenya. *Pan Afr Med J* 2015;**20**:108.
7. Akombi BJ, Agho KE, Merom D, Renzaho AM, Hall JJ. Child malnutrition in sub-Saharan Africa: a meta-analysis of demographic and health surveys (2006-2016). *PLoS One* 2017;**12**: e0177338.
8. Vorster HH, Kruger A. Poverty, malnutrition, underdevelopment and cardiovascular disease: a South African perspective. *Cardiovasc J Afr* 2007;**18**:321–24.
9. Benzekri NA, Sambou J, Diaw B *et al*. High prevalence of severe food insecurity and malnutrition among HIV-infected adults in Senegal, West Africa. *PLoS One* 2015;**10**:e0141819.
10. Guwatudde D, Mutungi G, Wesonga R *et al*. The epidemiology of hypertension in Uganda: findings from the national non-communicable diseases risk factor survey. *PLoS One* 2015;**10**: e0138991.
11. Kavishe B, Biraro S, Baisley K *et al*. High prevalence of hypertension and of risk factors for non-communicable diseases (NCDs): a population based cross-sectional survey of NCDS and HIV infection in Northwestern Tanzania and Southern Uganda. *BMC Med* 2015;**13**:126.
12. Ataklte F, Erqou S, Kaptoge S, Taye B, Echouffo-Tcheugui JB, Kengne AP. Burden of undiagnosed hypertension in sub-saharan Africa: a systematic review and meta-analysis. *Hypertension* 2015;**65**:291–98.
13. Lule SA, Elliott AM, Smeeth L, Webb EL. Is birth weight associated with blood pressure among African children and adolescents? A systematic review. *J Dev Orig Health Dis* 2018;**9**:270–80.
14. Noubiap JJ, Essouma M, Bigna JJ, Jingi AM, Aminde LN, Nansseu JR. Prevalence of elevated blood pressure in children and adolescents in Africa: a systematic review and meta-analysis. *Lancet Public Health* 2017;**2**:e375–86.
15. Delnord M, Blondel B, Zeitlin J. What contributes to disparities in the preterm birth rate in European countries? *Curr Opin Obstet Gynecol* 2015;**27**:133–42.
16. Garner P, Kramer MS, Chalmers I. Might efforts to increase birthweight in undernourished women do more harm than good? *Lancet* 1992;**340**:1021–23.
17. Christian P, Khatry SK, West KP Jr. Antenatal anthelmintic treatment, birthweight, and infant survival in rural Nepal. *Lancet* 2004;**364**:981–83.
18. Elliott AM, Kizza M, Quigley MA *et al*. The impact of helminths on the response to immunization and on the incidence of infection and disease in childhood in Uganda: design of a randomized, double-blind, placebo-controlled, factorial trial of deworming interventions delivered in pregnancy and early childhood [ISRCTN32849447]. *Clin Trials* 2007;**4**:42–57.
19. Webb EL, Mawa PA, Ndibazza J *et al*. Effect of single-dose anthelmintic treatment during pregnancy on an infant's response to immunisation and on susceptibility to infectious diseases in infancy: a randomised, double-blind, placebo-controlled trial. *Lancet* 2011;**377**:52–62.
20. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics* 2004; **114**:555–76.
21. Falkner B, Daniels SR. Summary of the fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Hypertension* 2004;**44**:387–88.
22. Leroy JL. zscore06: Stata command for the calculation of anthropometric z-scores using the 2006 WHO child growth standards, 2011. http://www.ifpri.org/staffprofile/jef-leroy.
23. WHO Multicentre Growth Reference Study Group. WHO child growth standards based on length/height, weight and age. *Acta Paediatr Suppl* 2006;**450**:76–85.
24. Law CM, Shiell AW. Is blood pressure inversely related to birthweight? The strength of evidence from a systematic review of the literature. *J Hypertens* 1996;**14**:935–41.
25. Huxley RR, Shiell AW, Law CM. The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. *J Hypertens* 2000;**18**:815–31.
26. Hardy R, Sovio U, King VJ *et al*. Birthweight and blood pressure in five European birth cohort studies: an investigation of confounding factors. *Eur J Public Health* 2006;**16**:21–30.
27. Huxley R, Neil A, Collins R. Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? *Lancet* 2002;**360**:659–65.
28. Griffiths PL, Sheppard ZA, Johnson W, Cameron N, Pettifor JM, Norris SA. Associations between household and neighbourhood socioeconomic status and systolic blood pressure among urban South African adolescents. *J Biosoc Sci* 2012;**44**:433–58.
29. Kagura J, Adair LS, Munthali RJ, Pettifor JM, Norris SA. Association between early life growth and blood pressure trajectories in black South African children. *Hypertension* 2016;**68**:1123–31.
30. Law CM, Shiell AW, Newsome CA *et al*. Fetal, infant, and childhood growth and adult blood pressure: a longitudinal study from birth to 22 years of age. *Circulation* 2002;**105**:1088–92.
31. Chiolero A, Paradis G, Madeleine G, Hanley JA, Paccaud F, Bovet P. Birthweight, weight change, and blood pressure during childhood and adolescence: a school-based multiple cohort study. *J Hypertens* 2011;**29**:1871–79.
32. Cournil A, Coly AN, Diallo A, Simondon KB. Enhanced postnatal growth is associated with elevated blood pressure in young Senegalese adults. *Int J Epidemiol* 2009;**38**:1401–10.
33. Bowers K, Liu G, Wang P *et al*. Birthweight, postnatal weight change, and risk for high blood pressure among Chinese children. *Pediatrics* 2011;**127**:e1272–79.
34. Kristman V, Manno M, Cote P. Loss to follow-up in cohort studies: how much is too much? *Eur J Epidemiol* 2004;**19**:751–60.
35. Millard JD, Muhangi L, Sewankambo M, Ndibazza J, Elliott AM, Webb EL. Assessing the external validity of a randomized controlled trial of anthelminthics in mothers and their children in Entebbe, Uganda. *Trials* 2014;**15**:310.

5.5 Supplementary figures

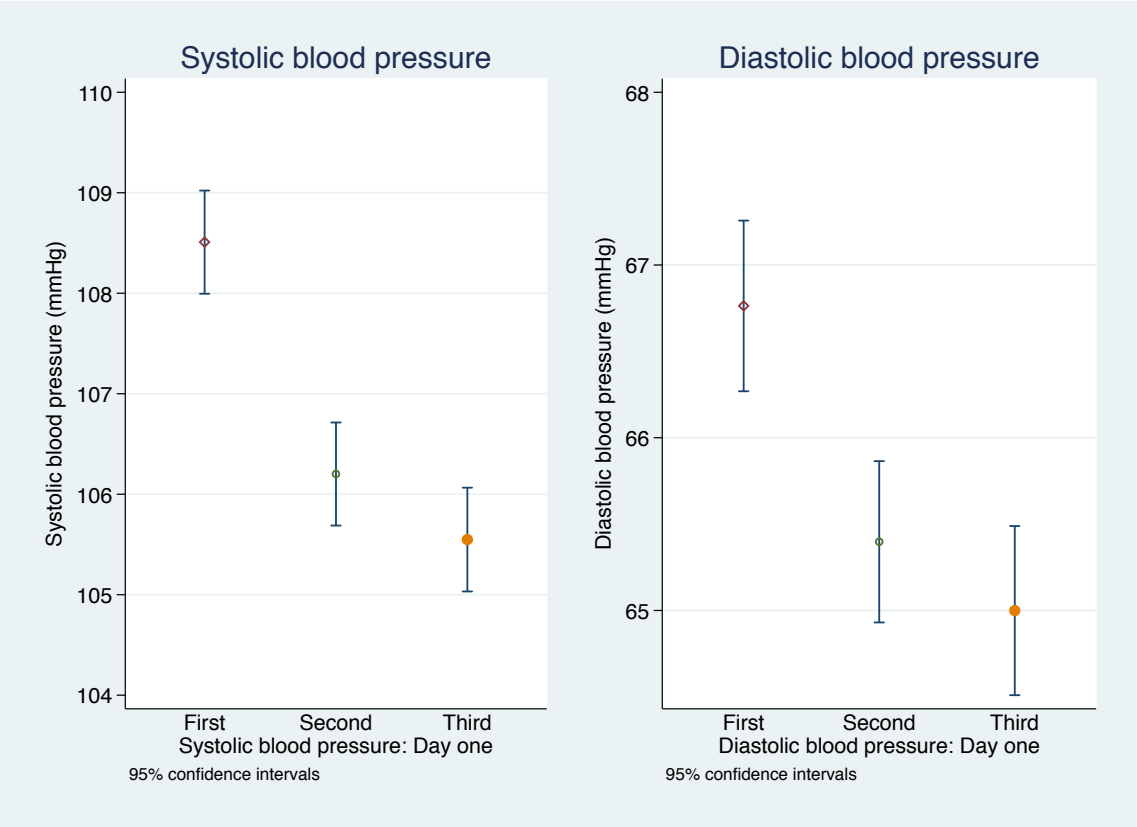

Figure S1: Means and their 95% confidence intervals for the three day-one BP measurements

5.6 Supplementary tables

Table S1: Characteristics of the Entebbe Mother and Baby Study participants enrolled and not enrolled into the blood pressure study (N=2345)

Mothers' characteristics during pregnancy	Participated (n=1119)		Did not participate (n=1226)	
	Number	Percentage/Mean (SD)	Number	Percentage/Mean (SD)
Age (years)*	1119	24.4 (5.5)	1226	23.1 (5.1)
Parity (number of children) *	1119	3.0 (1.8)	1226	2.7 (1.7)
Body mass index*	1110	24.1 (3.4)	1208	24.0 (3.1)
Household SES	1104	3.8 (1.2)	1197	3.5 (1.2)
Education level				
None	28	2.5	58	4.7
Primary	542	48.5	650	53.1
Senior	438	39.2	433	35.4
Tertiary	109	9.8	83	6.8
Marital status				
Single	116	10.4	191	15.6
Married/cohabiting	967	86.5	999	81.5
Separated/widowed	35	3.1	36	2.9
Area or residence				
Urban	770	69.6	831	68.9
Rural	336	30.4	376	31.2
Infections				
Asymptomatic malaria	109	9.9	139	11.6
Schistosomiasis	204	18.4	217	17.7
Hookworm	450	40.5	575	47.0
Ascaris	28	2.5	26	2.1
Trichuris	97	8.7	109	8.9
Trichostrongylus	11	1.0	11	0.8
Trial intervention 1				
Placebo	566	50.6	609	49.7
Albendazole	553	49.4	617	50.3
Trial intervention 2				
Placebo	564	50.4	611	49.8
Praziquantel	555	49.6	615	50.2
Participants' characteristics				
Birthweight (Kg)*	932	3.2 (0.5)	964	3.1 (0.5)
Sex				
Males	583	52.1	628	51.3
Birth type				
Singleton	1100	98.3	1190	97.1
Multiple	19	1.7	36	2.9
Feeding at 6 weeks of age				
Exclusive Breastfeeding	748	67.6	724	65.3
Mixed feeding	344	31.1	377	34.0
Weaned	14	1.3	7	0.6
HIV status				
Unexposed	1001	89.5	1064	86.8
Exposed not infected	100	8.9	126	10.3
Infected	18	1.6	36	2.9
Birth season				
Wet	651	58.2	683	55.9
Dry	468	41.8	539	44.1
Place of birth				
Entebbe Hospital	824	73.7	856	70.3
Home	120	10.7	144	11.8
Others	174	15.6	218	17.9
Mode of delivery				
Normal	1005	90.0	1111	91.1
Caesarean section	103	9.2	94	7.7
Instrumentation	9	0.8	15	1.2
Trial intervention 3				
Placebo	553	50.0	453	49.8
Albendazole	554	50.1	456	50.2

[*] Mean value with standard deviation (SD) presented
Percentages may total to ± 100 due to rounding
Household socioeconomic status (SES) was a composite variable and could take values 1 (low) to 6 (high)
Missing data for (a) mother's characteristics: body mass index 27; education 4; household SES 44; marital status 1; place of residence 32; asymptomatic malaria 43; schistosomiasis 9; hookworm 9; ascaris 9; trichuris 9; trichostrongylus 9; (b) child's characteristics: sex 2; birth weight 449; feeding status 131; birth season 4; place of delivery 9; mode of delivery 8; trial intervention 329.

Table S2: Crude and adjusted sensitivity analysis for the effect of birth weight on blood pressure among Entebbe Mother and Baby Study adolescents

Blood pressure Sensitivity analysis	Number	Mean birth weight (Kg)	Mean BP (mmHg)	Crude association		Adjusted association			
				β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)[3]	P-value
Lowest birthweight (1.26 kg)									
Systolic BP	1119	2.86	105.87	0.27 (-0.30, 0.84)	0.353	-0.04 (-0.80, 0.72)[1]	0.913	-0.53 (-1.26, 0.20)	0.152
Diastolic BP	1119	2.86	65.20	0.26 (-0.24, 0.76)	0.313	0.25 (-0.36, 0.86)[2]	0.423	-0.02 (-0.61, 0.57)	0.955
Largest birthweight (5.5 kg)									
Systolic BP	1119	3.57	105.87	0.10 (-0.40, 0.59)	0.692	0.07 (-0.58, 0.71)[1]	0.834	-0.24 (-0.85, 0.37)	0.438
Diastolic BP	1119	3.57	65.20	0.08 (-0.36, 0.51)	0.734	0.03 (-0.49, 0.56)[2]	0.905	-0.16 (-0.67, 0.34)	0.525

β; linear regression coefficient: mean difference in blood pressure measured in mmHg per 1kg increase in birth weight, CI; confidence interval

[1] Adjusted for maternal factors at enrolment (age, household socioeconomic status, body mass index, asymptomatic malaria, education, parity) and child factors (sex, age, family history of blood pressure)

[2] Adjusted for maternal factors at enrolment (age, household socioeconomic status, body mass index, asymptomatic malaria, education, parity) and child factors (sex, age, asymptomatic malaria, family history of blood pressure)

[3] Additionally adjusted for current weight

Table S3: Crude and adjusted relationship between birth weight categories and blood pressure among early adolescents in the Entebbe Mother and Baby Study

Birth weight (kg)	Number (%)	Mean (mmHg)	Crude β (95% CI)	P-value	Adjusted β (95% CI)	P-value	Adjusted β (95% CI)[3]	P-value
a) Systolic blood pressure								
<2.50	65 (7.0)	105.73	0.09 (-2.05, 2.24)		0.30 (-1.87, 2.47)[1]		0.86 (-1.19, 2.90)	
2.50-2.99	207 (22.2)	105.36	-0.28 (-1.65, 1.09)		-0.07 (-1.50, 1.35)[1]		0.30 (-1.04, 1.64)	
3.00-3.49	405 (43.5)	105.65	Reference		Reference		Reference	
≥3.50	255 (27.4)	106.82	1.18 (-0.10, 2.46)	0.208	0.78 (-0.56, 2.11)[1]	0.666	-0.06 (-1.33, 1.20)	0.832
b) Diastolic blood pressure								
<2.50	65 (7.0)	64.81	-0.34 (-2.22, 1.53)		0.17 (-1.64, 1.98)[2]		-0.17 (-2.04, 1.70)	
2.50-2.99	207 (22.2)	64.69	-0.46 (-1.66, 0.74)		-0.14 (-1.34, 1.06)[2]		-0.38 (-1.62, 0.86)	
3.00-3.49	405 (43.5)	65.15	Reference		Reference		Reference	
≥3.50	255 (27.4)	65.91	0.75 (-0.36, 1.88)	0.291	-0.10 (-1.23, 1.02)[2]	0.987	0.19 (-0.97, 1.35)	0.879

β; linear regression coefficient: mean difference in BP compared to adolescents weighing 3.00-3.49kg at birth

Percentages may total to ± 100 due to rounding

[1] Adjusted for maternal factors at enrolment (age, Household socio-economic status, body mass index, asymptomatic malaria, education, parity,) and child's factors (sex, age, family history of BP and place of birth)

[2] Adjusted for maternal factors at enrolment ((age, household socioeconomic status, body mass index, asymptomatic malaria, education, parity) and child's factors (sex, age, family history of blood pressure)

[3] Additionally adjusted for current weight current

Table S4 Sensitivity analysis of the association between postnatal weight gain and blood pressure among 10 and 11-year-old adolescents in the Entebbe Mother and Baby Study

Postnatal growth	Numbers	Crude β (95% CI)	P-value	Adjusted β (95% CI)	P-value
Lowest change in weight					
a) Systolic blood pressure					
Δ WAZ Birth-0.5 years	1119	0.33 (0.11, 0.55)	0.004	0.29 (0.04, 0.54)[1]	0.024
Δ WAZ 0.5-1 year	1119	0.46 (0.14, 0.78)	0.005	0.39 (-0.17, 0.95)[1]	0.172
Δ WAZ 1-2 years	1119	0.08 (-0.24, 0.40)	0.622	0.12 (-0.45, 0.69)[1]	0.679
Δ WAZ 2-5 years	1119	-0.11 (-0.42, 0.21)	0.506	-0.17 (-0.49, 0.15)[1]	0.300
Δ WAZ Birth-5 years	**1119**	**0.26 (0.03, 0.50)**	**0.030**	**0.32 (0.05, 0.59)[1]**	**0.019**
b) Diastolic blood pressure					
Δ WAZ Birth-0.5 years	1119	0.38 (0.19, 0.57)	<0.001	0.47 (0.25, 0.69)[2]	<0.001
Δ WAZ 0.5-1 year	1119	0.44 (0.16, 0.72)	0.002	0.33 (-0.16, 0.82)[2]	0.184
Δ WAZ 1-2 years	1119	0.01 (-0.28, 0.29)	0.968	-0.09 (-0.56, 0.43)[2]	0.787
Δ WAZ 2-5 years	1119	-0.03 (-0.31, 0.24)	0.808	-0.07 (-0.35, 0.22)[2]	0.641
Δ WAZ Birth- 5 years	**1119**	**0.27 (0.06, 0.48)**	**0.011**	**0.40 (0.16, 0.64)[2]**	**0.001**
Highest change in weight					
a) Systolic blood pressure					
Δ WAZ Birth-0.5 years	1119	0.29 (0.09, 0.49)	0.005	0.25 (0.02, 0.48)[1]	0.032
Δ WAZ 0.5-1 year	1119	-0.17 (-0.36, 0.03)	0.093	0.20 (-0.18, 0.57)[1]	0.300
Δ WAZ 1-2 years	1119	-0.05 (-0.33, 0.22)	0.711	0.58 (0.09, 1.07)[1]	0.020
Δ WAZ 2-5 years	1119	0.16 (-0.12, 0.44)	0.267	0.12 (-0.17, 0.41)[1]	0.410
Δ WAZ Birth -5 years	**1119**	**0.26 (0.05, 0.48)**	**0.018**	**0.33 (0.08, 0.58)[1]**	**0.010**
b) Diastolic blood pressure					
Δ WAZ Birth-0.5 years	1119	-0.05 (-0.26, 0.15)	0.607	0.08 (-0.16, 0.32)[2]	0.499
Δ WAZ 0.5-1 year	1119	-0.20 (-0.38, -0.03)	0.019	0.04 (-0.28, 0.37)[2]	0.791
Δ WAZ 1-2 years	1119	-0.12 (-0.37, 0.12)	0.322	0.36 (-0.07, 0.79)[2]	0.099
Δ WAZ 2-5 years	1119	0.18 (-0.07, 0.43)	0.158	0.16 (-0.10, 0.41)[2]	0.232
Δ WAZ **Birth-5 years**	**1119**	**0.19 (-0.01, 0.38)**	**0.058**	**0.18 (-0.05, 0.40)[2]**	**0.126**

Δ WAZ; change in weight-for-age-Z scores, β; linear regression coefficient: mean difference in blood pressure measured in mmHg per 1 unit increase in ΔWAZ

[1] Each growth period was adjusted for the earlier postnatal growth period, maternal factors at enrolment (age, household socioeconomic status, body mass index, asymptomatic malaria, education, parity) and child's factors (sex, age, feeding status at six weeks, family history of blood pressure)

[2] Each growth period was adjusted for the earlier postnatal growth period, maternal factors at enrolment (age, household socioeconomic status, body mass index, asymptomatic malaria, education, parity) and child's factors (sex, age, feeding status at six weeks, asymptomatic malaria, family history of blood pressure)

5.7 Supplementary tables (unpublished data)

Table S5: Correlation between early life (infancy or childhood) anthropometric parameters Z-scores and adolescents' anthropometric parameters Z-scores in the Entebbe Mother and Baby Study

	Current Weight	Current height	Current waist circumference
Age	**Pearson correlation**	**Pearson correlation**	**Pearson correlation**
At birth			
Weight	0.2410	0.1877	0.1747
Six weeks			
Weight	0.2103	0.1591	0.1820
Height/length	0.1011	0.1323	0.0843
Ten weeks			
Weight	0.2474	0.2026	0.2199
Six months			
Weight	0.4266	0.3418	0.3395
Nine months			
Weight	0.4544	0.3641	0.3523
One year			
Weight	0.5056	0.4345	0.4060
Height	0.3923	0.5059	0.2631
Two years			
Weight	0.5753	0.4879	0.4754
Height	0.4826	0.6387	0.3228
Three years			
Weight	0.5992	0.5042	0.4747
Height	0.5327	0.6779	0.3703
Four years			
Weight	0.6437	0.5354	0.5164
Height	0.5127	0.6705	0.3586
Five years			
Weight	0.6980	0.5812	0.5488
Height	0.5091	0.6779	0.3463
Six years			
Weight	0.7386	0.6120	0.5574
Height	0.6194	0.7633	0.4028
Seven years			
Weight	0.7470	0.6001	0.5937
Height	0.4299	0.5601	0.2777
Eight years			
Weight	0.7584	0.6181	0.6030
Height	0.5073	0.6991	0.3013
Nine years			
Weight	0.8177	0.6155	0.6664
Height	0.5689	0.7583	0.3614
Current			
Weight	-	0.7281	0.7331
Height	0.728	-	0.446
Waist	0.733	0.446	-

Current refers to age at blood pressure measurement (10 or 11 years)
Strong ($r \geq 0.5$) or moderate ($0.3 \leq r > 0.5$) correlations

Table S6: Showing the crude association between birth weight and blood pressure, and the adjusted associations with adjustment by each potential confounder with and without the forced variables

Adjusting factor	[1]Crude β (95% CI)	P-value	[2]Adjusted β (95% CI)	P-value	[3]Adjusted β (95% CI)	P-value
Systolic BP						
Mother						
Age	0.73 (-0.33, 1.80)	0.178	0.64 (-0.45, 1.73)	0.248	0.53 (-0.56, 1.62)	0.338
Household SES	0.71 (-0.36, 1.78)	0.196	0.64 (-0.44, 1.71)	0.248	0.55 (-0.53, 1.63)	0.315
Parity	0.73 (-0.33, 1.80)	0.178	0.72 (-0.36, 1.80)	0.193	0.62 (-0.47, 1.70)	0.263
Body mass index	0.71 (-0.36, 1.78)	0.194	0.34 (-0.75, 1.43)	0.539	0.27 (-0.83, 1.36)	0.632
Education status	0.75 (-0.32, 1.82)	0.169	0.59 (-0.48, 1.66)	0.279	0.50 (-0.57, 1.58)	0.358
Asymptomatic malaria	0.74 (-0.33, 1.81)	0.176	0.78 (-0.30, 1.86)	0.156	0.71 (-0.37, 1.79)	0.197
Participant						
Age	0.73 (-0.33, 1.80)	0.178	0.65 (-0.41, 1.71)	0.230	0.65 (-0.42, 1.72)	0.234
Sex	0.73 (-0.33, 1.80)	0.178	0.73 (-0.34, 1.80)	0.183	0.65 (-0.42, 1.72)	0.234
Place of birth	0.73 (-0.33, 1.80)	0.178	0.70 (-0.37, 1.78)	0.201	0.61 (-0.47, 1.69)	0.267
Family history of BP	0.73 (-0.33, 1.80)	0.178	0.66 (-0.41, 1.73)	0.227	0.55 (-0.52, 1.62)	0.311
Current weight	0.73 (-0.33, 1.80)	0.178	-0.87 (-1.88, 0.14)	0.093	-0.92 (-1.94, 0.10)	0.077
All factors[4]	0.73 (-0.33, 1.80)	0.178	-0.86 (-1.93, 0.21) [5]	0.116	-0.93 (-2.00, 0.15)	0.092
Diastolic BP						
Mother						
Age	0.66 (-0.27, 1.59)	0.164	0.52 (-0.42, 1.47)	0.278	0.48(-0.47, 1.42)	0.325
Household SES	0.70 (-0.24, 1.64)	0.144	0.62 (-0.32, 1.56)	0.196	0.59 (-0.35, 1.53)	0.217
Parity	0.66 (-0.27, 1.59)	0.164	0.61 (-0.34, 1.55)	0.207	0.57 (-0.38, 1.51)	0.240
Body mass index	0.65 (-0.28, 1.58)	0.171	0.43 (-0.52, 1.38)	0.376	0.42 (-0.53, 1.37)	0.385
Education status	0.68 (-0.25, 1.62)	0.152	0.60 (-0.34, 1.54)	0.212	0.57 (-0.37, 1.51)	0.234
Asymptomatic malaria	0.69 (-0.25, 1.63)	0.150	0.68 (-0.27, 1.62)	0.161	0.67 (-0.27, 1.62)	0.163
Participant						
Age	0.66 (-0.27, 1.59)	0.164	0.58 (-0.35, 1.50)	0.220	0.64 (-0.29, 1.57)	0.180
Sex	0.66 (-0.27, 1.59)	0.164	0.72 (-0.22, 1.65)	0.133	0.64 (-0.29, 1.57)	0.180
Place of birth	0.66 (-0.27, 1.59)	0.164	0.71 (-0.22, 1.65)	0.136	0.69 (-0.25, 1.63)	0.150
Current weight	0.66 (-0.27, 1.59)	0.164	-0.53 (-1.43, 0.38)	0.256	-0.48 (-1.40, 0.43)	0.300
Family history of BP	-0.53 (-1.43, 0.38)	0.256	0.62 (-0.32, 1.56)	0.195	0.58 (-0.36, 1.52)	0.227
Asymptomatic malaria	0.79 (-0.15, 1.72)	0.099	0.73 (-0.21, 1.66)	0.126	0.71 (-0.22, 1.65)	0.135
All factors[4]	0.66 (-0.27, 1.59)	0.164	0.44 (-0.56, 1.45) [5]	0.388	-0.43 (-0.57, 1.43)	0.402

β; Linear regression coefficient, Birth weight analysed as a continuous variable, P value from the partial F test

[1] Crude linear regression coefficient excluding missing data for the adjusting factor

[2] Adjusted for a given factor

[3] Additionally, adjusted for forced variables (sex and current age)

[4] Adjusting for all the above factors

[5] Forced variables not included in this analysis

Table S7: Crude and adjusted relationship between birth weight categories and blood pressure among early adolescents in the Entebbe Mother and Baby Study

Birth weight (kg)	Number (%)	Mean (mmHg)	Crude β (95% CI)	P-value	Adjusted β (95% CI)	P-value	[1] Adjusted β (95% CI)	P-value
a) Systolic blood pressure[2]								
<2.50	65 (7.0)	105.73	0.09 (-2.05, 2.24)		0.30 (-1.87, 2.47)		0.86 (-1.19, 2.90)	
2.50-2.99	207 (22.2)	105.36	-0.28 (-1.65, 1.09)		-0.07 (-1.50, 1.35)		0.30 (-1.04, 1.64)	
3.00-3.49	405 (43.5)	105.65	Reference		Reference		Reference	
≥3.50	255 (27.4)	106.82	1.18 (-0.10, 2.46)	0.208	0.78 (-0.56, 2.11)	0.666	-0.06 (-1.33, 1.20)	0.832
b) Diastolic blood pressure[3]								
<2.50	65 (7.0)	64.81	-0.34 (-2.22, 1.53)		0.17 (-1.64, 1.98)		-0.17 (-2.04, 1.70)	
2.50-2.99	207 (22.2)	64.69	-0.46 (-1.66, 0.74)		-0.14 (-1.34, 1.06)		-0.38 (-1.62, 0.86)	
3.00-3.49	405 (43.5)	65.15	Reference		Reference		Reference	
≥3.50	255 (27.4)	65.91	0.75 (-0.36, 1.88)	0.291	-0.10 (-1.23, 1.02)	0.987	0.19 (-0.97, 1.35)	0.879

β; linear regression coefficient

Percentages may total to ± 100 due to rounding

[1] additionally adjusted for current weight current

[2] Adjusted for maternal factors at enrolment (age, Household socio-economic status, body mass index, asymptomatic malaria, education, parity,) and child's factors (sex, age, family history of BP and place of birth)

[3] Adjusted for maternal factors at enrolment ((age, household socioeconomic status, body mass index, asymptomatic malaria, education, parity) and child's factors (sex, age, family history of blood pressure)

Table S8: Characteristics of the Entebbe Mother and Baby Study adolescents at the time of enrolment into the study on blood pressure, separately for males and females (N=1119)

	Overall		Males (n=583)		Females (n=536)	
Characteristics	**Mean/Median**	**SD/IQR**	**Mean/Median**	**SD/IQR**	**Mean/Median**	**SD/IQR**
Age (years)[1]	10.16	10.01-10.85	10.19	10.01-10.87	10.11	10.01-10.85
Weight (kg)	29.54	5.23	29.30	4.83	29.81	5.63
Height (cm)	135.66	6.91	134.90	6.99	136.50	6.73
BMI	15.97	1.88	16.03	1.72	15.90	2.04
Mean[2] systolic BP (mmHg)	105.87	8.22	105.88	7.47	105.87	8.96
Mean[2] diastolic BP (mmHg)	65.20	7.26	64.89	7.17	65.53	7.36

[1] Median and IQR reported

[2] Mean of the last two of the three consecutive measurements

BMI; body mass index, SD; standard deviation, IQR; inter quintile range, BP; blood pressure

5.8 Testing assumptions underlying linear regression analysis

Inference from linear regression analyses are contingent on certain assumptions being correct. Assumptions underlying linear regression are:

1. A linear relationship between the dependent variable (systolic or diastolic BP) and the main predictor variable (birth weight)
2. Observations are independent
3. Homoscedasticity: the true residual variance is constant
4. The true residuals are normally distributed

It is not common for all the assumptions underlying linear regression to hold. Departures from assumptions are less important for large datasets than for small ones. Graphical methods were used to investigate the assumptions underlying linear regression for the effect of birth weight on BP.

5.8.1 Scatter plots of blood pressure (dependent variable) against birth weight (predictor variable)

Figure 5.8-1 shows scatter plots of systolic and diastolic BP against birth weight, the plot is important for identifying heteroscedasticity, non-linearity and outliers.

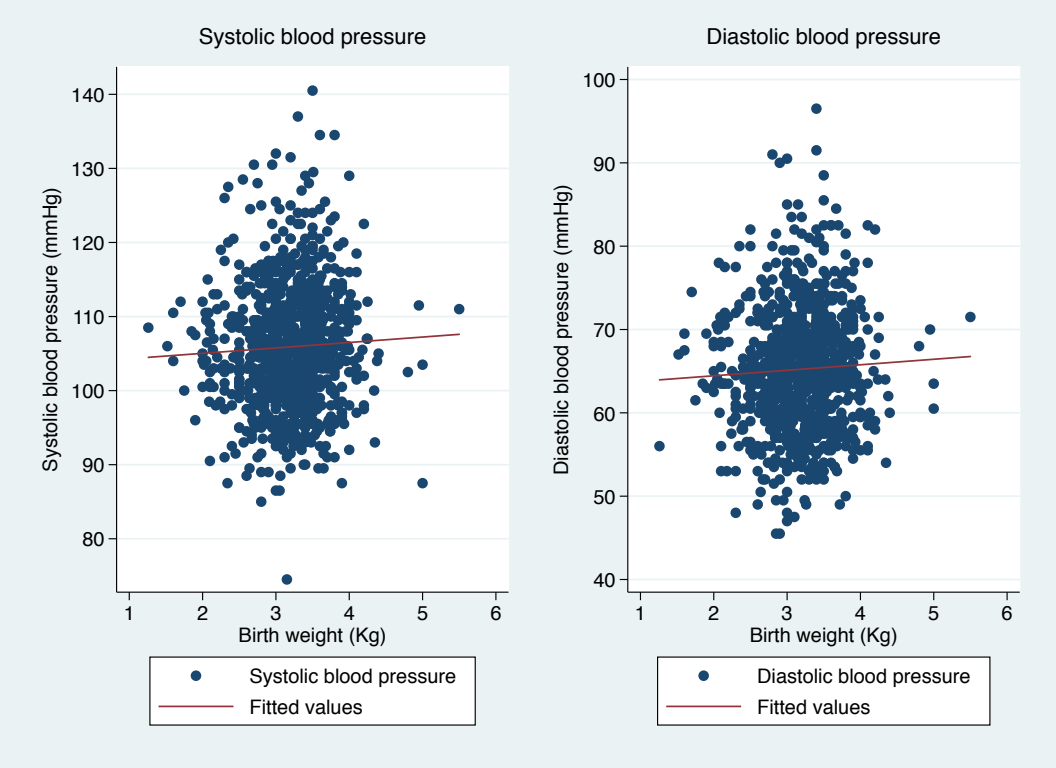

Figure 5.8-1: Relationship between blood pressure and birth weight

These plots show little suggestion of a non-linear relationship (assumption 1) for either systolic or diastolic BP. Both systolic and diastolic BP increase per unit increase in birth weight. The plots show that the scatters are consistent along the regression lines, evenly distributed and there are no obvious outliers.

To further investigate assumption 1, I examined whether a quadratic relationship might better explain any association between BP and birth weight than a linear relationship. Quadratic and linear relationships were fitted on the same plot. Fgure 5.8-2 shows results from both modelling approaches for systolic and for diastolic BP. The quadratic regression model describes a non-linear relationship between systolic or diastolic BP (Y variable) and birth weight (X variable). There was little evidence for a quadratic relationship between systolic BP and birth weight (p-value=0.779) and little evidence for a quadratic relationship between diastolic BP and birth weight (p-value=0.726). The data were consistent with a linear relationship between birth weight and BP (p-values for departure from linearity were 0.253 and 0.404 for systolic and diastolic BP respectively).

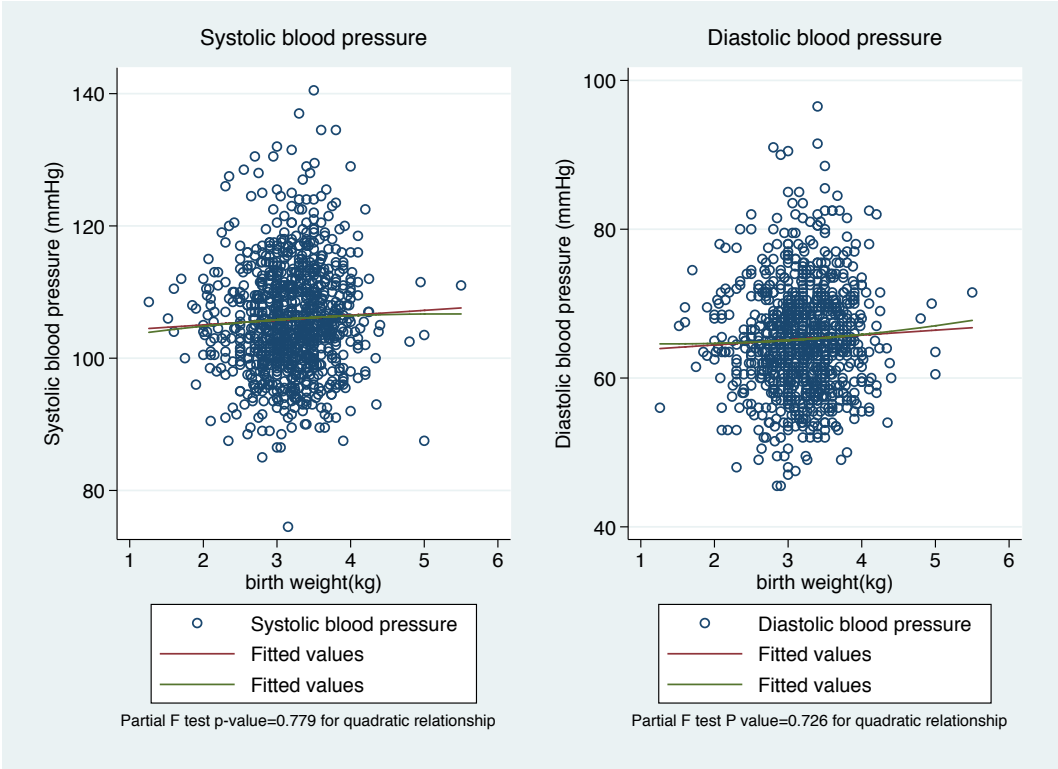

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Figure 5.8-2: Fitting both linear and quadratic relationships between blood pressure and birth weight

Assumption 2, “observations are independent” could not be tested graphically and there is no reason to think that the observations might not be independent.

5.8.2 Plots of residuals against birth weight (predictor) variables and against fitted values

Linear regression assumptions hold when the true residuals are normally distributed with constant variance (assumption 3). Figure 5.8-3 and Figure 5.8-4 show that the points are equally distributed above and below zero on the Y axis for both systolic and diastolic BP. The variance of residuals is constant across y and x. The distribution of residuals for systolic and diastolic BP are normally distributed. Figure 5.8-4 shows residuals plotted against fitted values in Figure 5.8-3

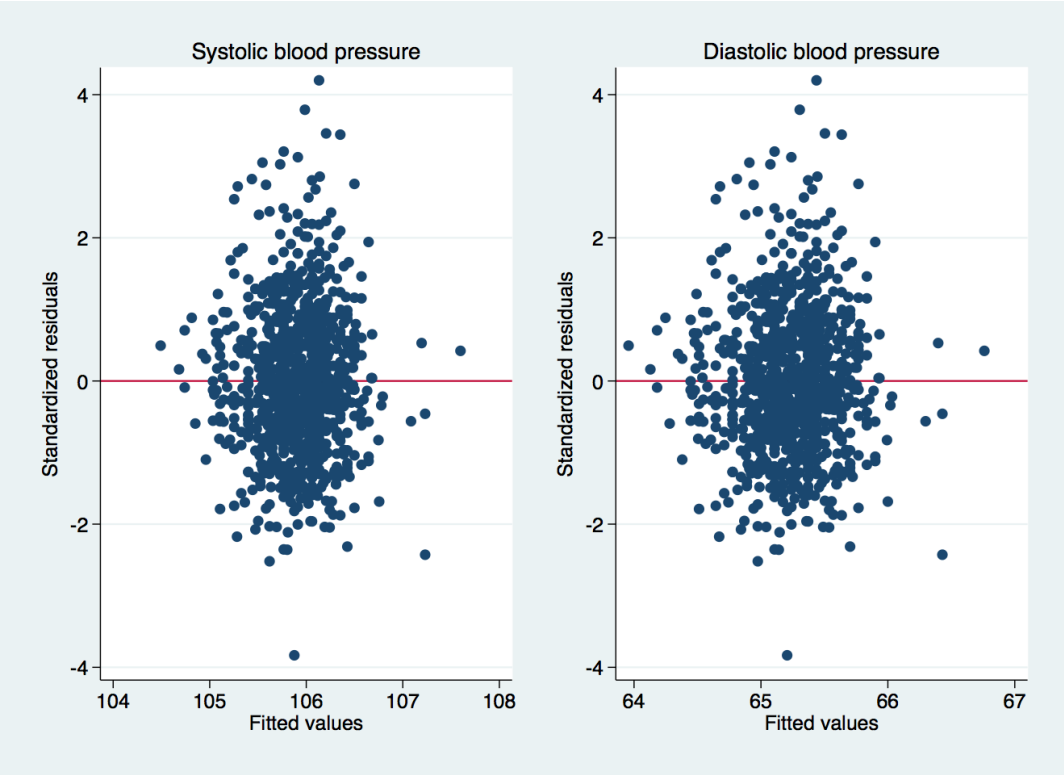

Figure 5.8-3: Scatter of standardized residuals versus fitted values

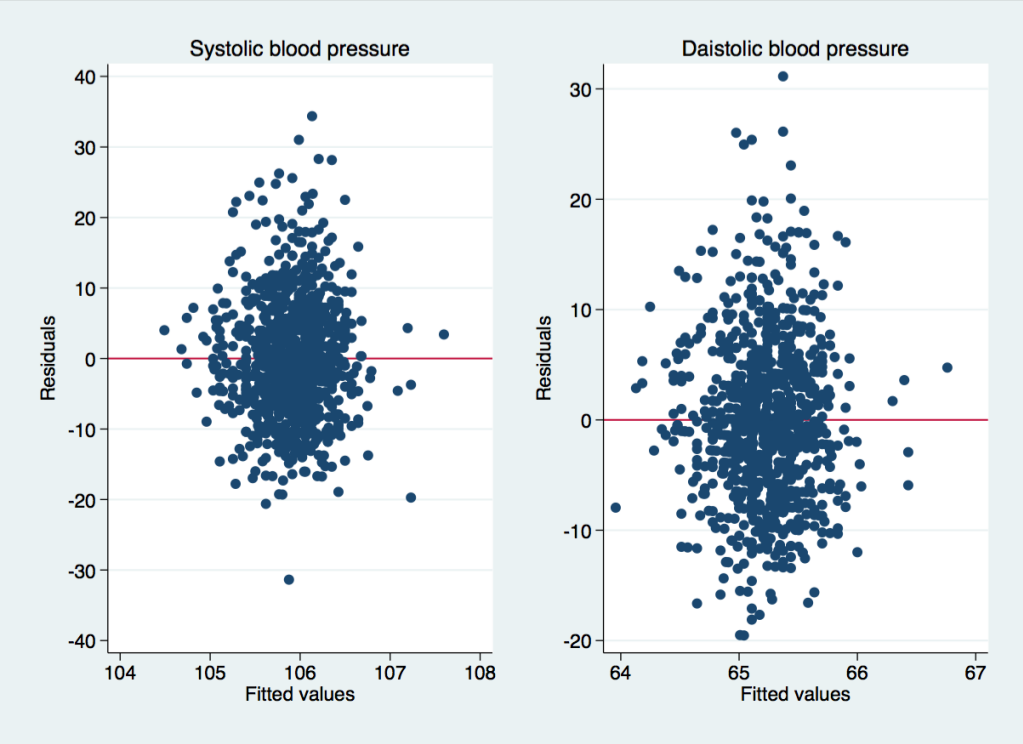

Figure 5.8-4: Scatter of residuals versus fitted values

5.8.3 Normal plot of residuals

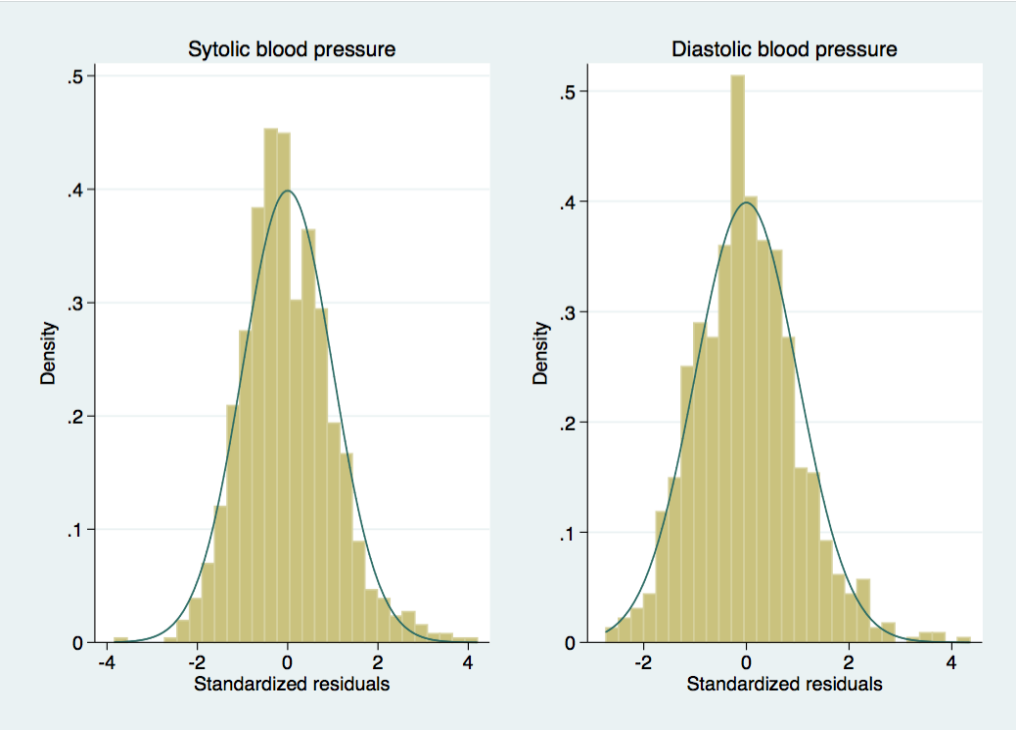

Figure 5.8-5: Histogram of blood pressure residuals

Figure 5.8-5 shows that the residuals come from a normal distribution and are independent from each other (assumption 4)

Chapter 6: Other environmental factors associated with blood pressure among Ugandan adolescents

6.1 Introduction

This chapter uses phenotypic data from the EMaBS birth cohort to answer thesis objective 4 which aims to identify other factors associated with BP among Ugandan adolescents.

6.2 Research paper 3: Blood pressure risk factors in early adolescents: Results from a Ugandan birth cohort

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	Abubaker Swaib Lule
Principal Supervisor	Emily Webb
Thesis Title	Investigating the impact of early-life, life-course and genetic factors on blood pressure among young Africans

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

**If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.*

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Journal of Human Hypertension
Please list the paper's authors in the intended authorship order:	Swaib A. Lule Benigna Namara, Helen Akurut, Lawrence Lubyayi, Margaret Nampijja, Florence Akello, Josephine Tumusiime, Judith C. Aujo, Gloria Oduru, Alexander Mentzer, Liam Smeeth, Alison M. Elliott, Emily L. Webb
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceptualised and designed the study, drafted the study documents (including protocol, standard operating procedures, questionnaires, consent and assent information sheets and forms), conducted the study (including data collection, data

	management and cleaning), performed statistical analysis, interpreted the findings and drafted, revised and submitted the final manuscript

Student Signature: _____ **Date:** 2/10/2018

Supervisor Signature: _____ **Date:** 2/10/2018

Blood pressure risk factors in early adolescents: results from a Ugandan birth cohort

Swaib A. Lule[1,2], Benigna Namara[2], Helen Akurut[2], Lawrence Lubyayi[2], Margaret Nampijja[2], Florence Akello[2], Josephine Tumusiime[2], Judith C. Aujo[3], Gloria Oduru[2], Alexander J. Mentzer[4], Liam Smeeth[1], Alison M. Elliott[1,2], Emily L. Webb[1]

Author affiliations

[1]London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

[2]MRC/UVRI & LSHTM Uganda Research Unit, P.O. Box 49, Entebbe, Uganda

[3]Mulago Hospital, Department of Paediatrics, P.O. Box 7051, Kampala, Uganda

[4]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK

Correspondence: Dr Swaib Abubaker Lule, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK. swaiblule@yahoo.com

Conflicts of Interest: None declared. The sponsor had no role in the study design, collection, analysis and interpretation of the data, report writing and the decision to submit the manuscript for publication. The first author wrote the first draft of the manuscript and no form of payment was given for writing the manuscript.

Short title: Blood pressure risk factors in adolescents

Abstract

We aimed to investigate life-course factors associated with blood pressure (BP) among Ugandan adolescents. Between 9th April 2003 and 24th November 2005, 2,507 pregnant women from Entebbe municipality and Katabi sub-county were enrolled into a deworming trial. The resulting 2,345 live born offspring were followed to age 10 or 11 years, when between 20th May 2014 to 16th June 2016, BP was measured following standard protocols. Factors associated with BP were assessed using multivariable linear regression. BP was measured in 1,119 adolescents with a median age of 10.2 years. Mean systolic BP and diastolic BP was 105.9 mmHg (standard deviation (SD) 8.2) and 65.2 mmHg (SD 7.3), respectively. Maternal gestational body mass index (BMI), higher maternal education status and family history of hypertension were positively associated with adolescent BP. Childhood (age ≤5 years) malaria was associated with lower adolescent systolic BP. Factors measured at time of BP measurement positively associated with systolic BP were age, BMI, waist circumference and *Trichuris* (whipworm) infection; higher vegetable consumption was associated with lower systolic BP. Results for diastolic BP were similar, except higher fruit, rather than higher vegetable consumption was associated with lower diastolic BP and there was no association with waist circumference or *Trichuris* infection. In summary, life-course exposures were associated with adolescent BP in this tropical birth cohort. Malaria early in life could impact later BP. Interventions initiated early in life targeting individuals with family history of hypertension, aiming to reduce adiposity (in pregnancy and adolescence) and promoting fruit and vegetable consumption might contribute to reducing the risk of high BP and subsequent CVDs.

Keywords: blood pressure, risk factors, adolescents, birth cohort, Uganda

Summary

What is known about the topic?

- High blood pressure and cardiovascular diseases (CVDs) are increasing in Africa.
- Scarcity of data on BP risk factors among African children and adolescents.
- The risk factors for high BP may differ from those seen in high-income non-tropical settings.

What this paper adds?

- Malaria infection in childhood is associated with reduced blood pressure among adolescents. Effects of childhood malaria on later blood pressure may be partially mediated through chronic reduction in weight and height.
- Current infection with *Trichuris* is associated with increased blood pressure.
- Interventions during pregnancy, childhood and early adolescence could be vital in the prevention of high BP later in life.

Introduction

Once uncommon in Africa [1], high blood pressure (BP) and cardiovascular diseases (CVDs) have escalated on the continent over the last three decades [2], affecting populations at younger ages than in more affluent countries [3], The rising burden of high BP in Africa has been attributed to a transition from active to more sedentary lifestyles and a rise in unhealthy dietary practices [2]. Data on individual level BP risk factors in African adolescents and children is sparse.

Although high BP is less common in children and adolescents than in adults, it initiates early in life, persists into adulthood [4] and predicts adulthood hypertension [5]. Diagnosis of CVDs is uncommon until middle-age, yet its antecedents, mainly cardiovascular and metabolic changes, begin early in life [6]. Globally, the high BP burden in younger age groups has risen [7], with estimated prevalence of 1-25% among African children and adolescents [8].

Severe persistent high BP is associated with increased risk of stroke and heart failure [9]; treatment reduces long-term sequelae [9]. In children and adolescents, high BP is often asymptomatic and unnoticed, despite international recommendations for regular BP measurement from three years of age [10]. Hypertension diagnosis is commonly missed or inaccurately classified in children and adolescents [11]. Consequently, over 75% of high BP among children and adolescents remains undiagnosed worldwide [12].

Earlier studies, mainly in adults, have demonstrated the role of established risk factors for high BP such as obesity [13] and physical activity [14]. There is little literature on childhood and adolescent BP determinants from Africa; in particular the impact of childhood infections (of special importance in Africa) remains understudied and unknown.

Childhood and adolescence are opportune periods for high BP control or prevention before clinical manifestation of hypertension or related CVDs. Identification of life-course BP risk factors unique to Africa is needed for the development of appropriate BP control strategies. We used longitudinally collected data from the Entebbe Mother and Baby Study (EMaBS), a large tropical birth cohort, to describe factors associated with adolescent BP.

Methods

Study design, setting and population

This longitudinal observational study investigated perinatal and life-course factors associated with BP among adolescents born in Wakiso district, Uganda. The EMaBS was a randomised double-blind placebo-controlled factorial trial [trial number; ISRCTN32849447], designed to investigate effects of worms and their treatment in pregnancy and childhood on response to childhood vaccines and on infections [15].

The study was conducted in Entebbe municipality and Katabi sub-county (a peninsula on the northern shores of Lake Victoria). In 2003-2005, 2,507 women attending Entebbe Hospital antenatal clinic, in their second or third trimester were invited, enrolled and randomised to receive albendazole (400mg) or placebo and praziquantel (40 mg/kg) or placebo [15].

Data were collected prenatally from women and resulting 2,345 live-born offspring followed from birth. As previously described [16], at 15 months offspring were randomised to receive quarterly single-dose albendazole or placebo up to age five years. Children continued under follow-up (seen at routine annual visits and when sick) after trial completion. Between 20th May 2014 and 16th June 2016, additional data, including BP measurements, anthropometry, puberty, physical activity and diet were collected from 10 and 11-year-olds. Enrolment was postponed for those with malaria (fever with malaria parasites) or other illness until they were well after being treated by the study team. Adolescents participated once, on their first 10 or 11-year annual visit occurring during the study period.

Study procedures

Birth weight was measured and recorded immediately after birth in Entebbe hospital or from child health cards for deliveries conducted elsewhere [17]. Weight and height at 10/11 years were measured with scales (Seca, Hamburg, Germany) and stadiometers (Seca 213, Hamburg Germany), respectively. Waist circumference was measured to the nearest 0.1cm using a Seca tape measure (Seca 201, Hamburg, Germany). BMI was calculated as weight in kilograms (kg) divided by height squared (m^2). Trained clinicians examined and performed Tanner staging [18].

Whole-genome genotyping of 1,391 EMaBS samples was conducted at the Wellcome Trust Sanger Institute using Illumina HumanOmni2.5M-8 ('octo') Beadchip arrays, version 1.1 (Illumina Inc., San Diego, USA). Sickle-cell trait was imputed using a merged 1000 Genomes and African-specific reference panel [19].

For participants taking part in the BP study from the 21st January 2015 to 23rd December 2015, extra data on fat mass (FM), fat-free mass (FFM) and total body water mass (TBW) was collected by trained nurses using a segmental body composition analyser machine (SBCAM) (TANITA BC-418, TANITA Corporation, Tokyo Japan). Briefly, participants stood barefooted on the posterior electrode base while holding two anterior electrodes handles of the SBCAM. Fat mass index=FMI (kg)/height(m^2), fat-free mass index=FFMI (kg)/height(m^2) and total body water mass index=TBWI (kg)/height(m^2) were computed.

Stool and blood samples were collected from women at enrolment and annually from children. Stool was examined for helminth ova and *Strongyloides* larvae using Kato-Katz [20] and charcoal culture [21] methods, respectively. Blood was examined for malaria parasites using Leishman's stains [16]. Modified Knott's method [22] was used for *Mansonella perstans*. Maternal HIV status at enrolment and children's HIV status after 18 months of age were assessed using a rapid serial testing algorithm described elsewhere [21, 23]. In infancy, HIV status was determined using polymerase chain reaction [21].

At the 10 or 11-year annual visit, three BP measurements (at approximately five minute intervals) were taken after five minutes rest using automated devices (Omron M6), with appropriate sized cuffs [5], by trained nurses following standard protocols described elsewhere [17].

For clinical care purposes, means of the three systolic BP and three diastolic BP measurements were calculated and BP percentiles determined using Centre for Disease Control height charts and 2004 updated National Health and Nutrition Examination Survey BP tables specific for sex, age and height [5, 10]. Those with mean systolic BP or diastolic BP ≥95th percentile ("high BP") had their BP re-measured on up to two extra days, 1-2 weeks apart. "Pre-hypertension" was defined as systolic or diastolic BP ≥90th but <95th percentile. Those with persistent high BP on three different days were referred for specialist attention. Lifestyle modification was recommended for participants with systolic or diastolic BP ≥90th percentile.

For data analysis purposes, the means of the second and third systolic / diastolic BP readings on day-one were used: day-one second and third BP readings were lower than the first BP reading but similar to each other [17].

Ethical approval was granted by the Uganda Virus Research Institute Science and Ethics Committee; the Uganda National Council for Science and Technology; and the London

School of Hygiene and Tropical Medicine. Written informed assent and consent were obtained.

Statistical methods

Data were collected on pre-coded questionnaires and analysed with Stata 14.2 (College Station, TX, USA). Chi-squared tests (for categorical variables) and t-tests (for continuous variables) were used to compare characteristics of cohort members who participated and did not participate in the BP study.

Study outcomes were mean systolic BP and mean diastolic BP, based on the second and third day-one measurements. The decision was made to model these two continuous BP outcome variables rather than to dichotomise outcomes (for example, into normal versus hypertensive) since an analysis using these binary outcomes would be underpowered. Maternal, perinatal, and offspring life-course factors considered as exposures and potential confounders were: maternal and adolescent socio-demographic and anthropometric characteristics; EMaBS trial interventions (praziquantel or albendazole); sickle-cell trait; illnesses and infections from birth to time of BP measurement; and body composition, puberty stage, diet, sleep pattern and physical activity at time of BP measurement. Area of residence was grouped into urban versus rural area using zones based on topography and settlements generated from geographical positioning system data [24]. Household socioeconomic index was generated using principal components analysis of building materials, household size and items owned [23]. Birth season was dichotomised into dry (rainfall below monthly median) and wet (rainfall above monthly median) season. Malaria infection in childhood (age ≤5 years) was investigated as clinical malaria (history of fever within the last 48 hours or axillary temperature ≥37.5°C and parasitaemia) and asymptomatic malaria (parasitaemia without fever at any annual visit up to five years). Information on diet was obtained as the number of days in a typical week over the previous month for which a given food was consumed. Puberty was grouped into pre-pubertal (stage 1) or pubertal (stages 2-5) for breast or pubic hair development using Tanner methods [18].

Linear regression analysis was used. Data satisfied the assumptions for linear regression. Crude associations were examined for each covariate and a 20% significance level used for selecting covariates for multivariable models. Adolescents' sex, age and BMI were confounders *a priori*. Multivariable analysis followed a hierarchical approach adding factors sequentially (Figure 6.2-1).

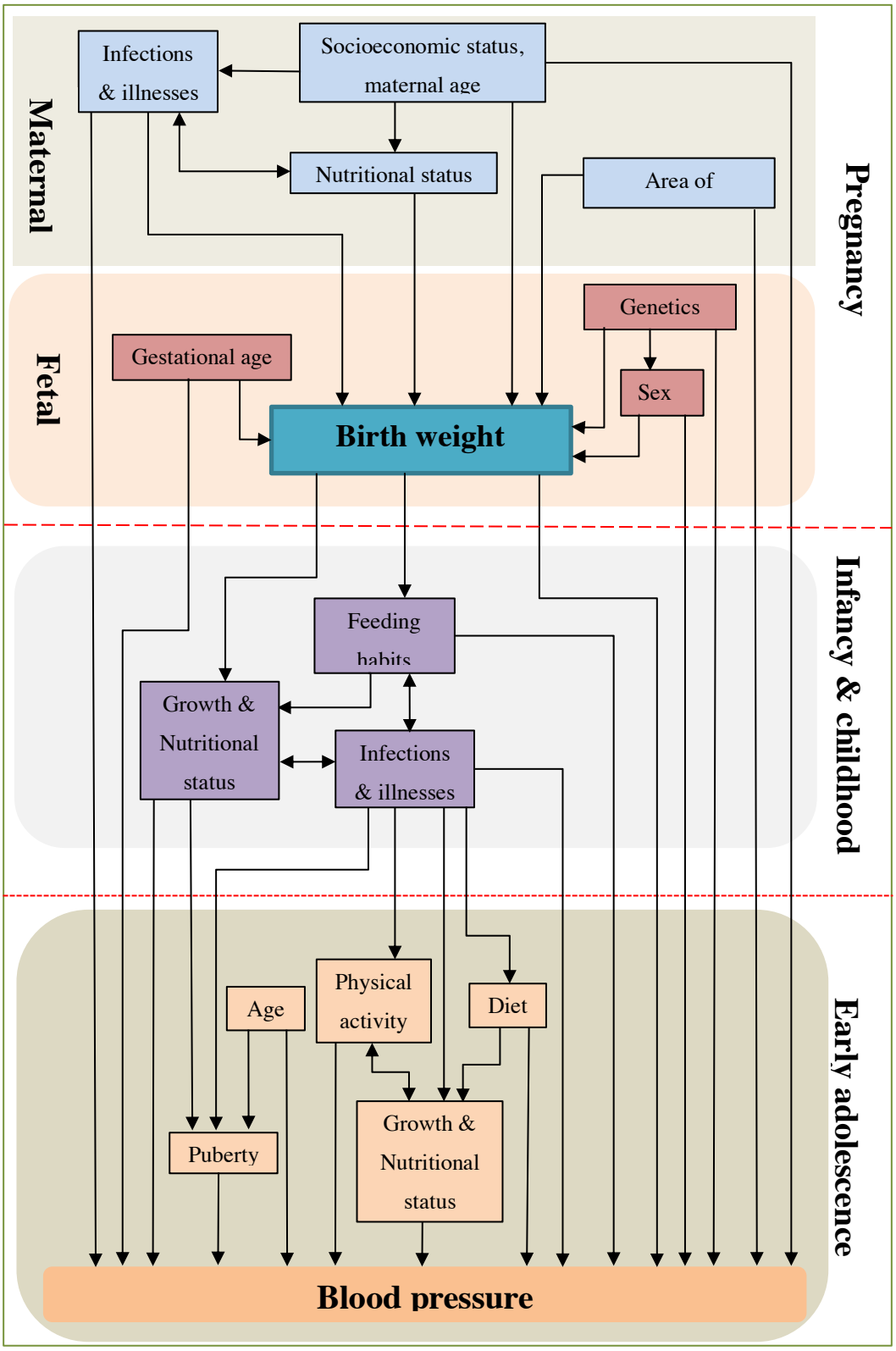

Figure 6.2-1 Conceptual framework

Because of a large proportion of missing data, puberty and body composition variables were not included in model building for other exposures but their effects were each adjusted for variables included in the final multivariable model. Multicollinearity was

assessed by considering the change in standard error, when potentially multicollinear variables were included in the same model.

The study was approved by the Research and Ethics Committee of the Uganda Virus Research Institute, the Uganda National Council for Science and Technology and the London School of Hygiene & Tropical Medicine. Consent and assent were obtained for study participation.

Results

Participant characteristics

A total of 1,119 EMaBS participants were enrolled into the BP study: 583 (52%) were males; 1,100 (98.3%) singletons; 18 (2%) HIV positive; and 344 (31%) mixed feeding by six weeks. EMaBS adolescents participating in the BP study were similar to non-participants, except that mothers of participants were more likely to be of higher education status or married/cohabiting; offspring were less likely to be HIV positive or of a multiple birth, details published earlier [17].

At age 10/11 (median participant age 10.2 years (interquartile range (IQR): 10.0-10.9)), 117 (11%) were attending boarding schools, 441 (72%) were pre-pubertal stage for pubic hair development and 178 (65%) of girls were pre-pubertal stage for breast development. Mean BMI was 15.8kg/m^2 (SD 1.9) and mean waist circumference 58.1cm (SD 4.9). Body composition data were available for 176 (16%) participants, with mean fat mass index 2.9kg/m^2 (SD 1.2), fat-free mass index 12.8kg/m^2 (SD 1.4) and total body water mass index 9.5kg/m^2 (SD 0.9).

Over the previous month, starchy staple foods, animal proteins, fruit, vegetables and sugar drinks were consumed on average for 6.9 days/week (SD 0.8), 2.2 days/week (SD 1.7), 3.0 days/week (SD 2.2), 3.4 days/week (SD 2.3), and 1.7 days/week (SD 2.1), respectively. Nearly all adolescents (98%) reported adding salt to cooked food.

Mean systolic BP was 105.9mmHg (SD 8.2) and mean diastolic BP was 65.2mmHg (SD 7.3). There was no difference in mean systolic BP (p-value=0.971) or diastolic BP (p-value=0.141) between males and females. None of the adolescents had had a prior BP measurement or high BP diagnosis.

Table 1: Factors investigated for association with systolic blood pressure among adolescents from the Entebbe Mother and Baby Study (N=1119)

Factors	Mean BP (SD)	Crude β (95% CI)	P-value	Adjusted β (95% CI)	P-value
Level 1: Maternal factors at enrolments					
Age (years)		0.06 (-0.03, 0.15)	0.178	0.02 (-0.07, 0.12)	0.604
Household SES (n=1104)		0.23 (-0.16, 0.63)	0.245		
Parity		0.04 (-0.23, 0.31)	0.751		
Body mass index (n=1110)		0.27 (0.13, 0.42)	<0.001	**0.26 (0.11, 0.40)**	**<0.001**
Education status					
None (n=28)	104.5 (8.7)	-0.54 (-3.65, 2.56)		**-0.62 (-3.77, 2.53)**	
Primary (n=542)	105.0 (7.7)	Reference		**Reference**	
Senior (n=438)	106.5 (8.2)	1.45 (0.42, 2.48)		**1.43 (0.39, 2.47)**	
Tertiary (n=109)	108.2 (9.8)	3.19 (1.51, 4.87)	<0.001	**3.14 (1.45, 4.84)**	**<0.001**
Marital status					
Single (n=116)	104.7 (7.6)	-1.34 (-2.92, 0.25)			
Married/cohabiting (n=967)	106.0 (8.3)	Reference			
Separated/widowed (n=35)	105.3 (6.1)	-0.78 (-3.56, 1.99)	0.229		
Area of residence					
Urban (n=770)	106.0 (8.3)	Reference			
Rural (n=336)	105.5 (8.0)	-0.47 (-1.52, 0.59)	0.386		
Alcohol use					
No (n=775)	105.8 (8.4)	Reference			
Yes (n=343)	106.0 (7.8)	0.15 (-0.90, 1.19)	0.781		
Infections					
HIV					
Uninfected (n=1002)	106.0 (8.3)	Reference		Reference	
Infected (n=117)	104.8 (7.2)	-1.17 (-2.74, 0.41)	0.146	-0.88 (-2.48, 0.72)	0.279
Asymptomatic malaria					
Uninfected (n=991)	105.8 (8.2)	Reference			
Infected (n=109)	106.2 (8.6)	0.42 (-1.20, 2.05)	0.609		
Schistosomiasis					
Uninfected (n=908)	105.8 (8.3)	Reference			
Infected (n=204)	106.2 (7.9)	0.35 (-0.90, 1.61)	0.578		
Hookworm					
Uninfected (n=662)	105.8 (8.1)	Reference			
Infected (n=450)	105.9 (8.4)	0.10 (-0.89, 1.09)	0.844		
Ascaris					
Uninfected (n=1084)	105.9 (8.3)	Reference			
Infected (n=28)	105.7 (6.7)	-0.17 (-3.27, 2.92)	0.912		
Intervention one					
Placebo (n=566)	105.5 (8.2)	Reference		Reference	
Albendazole (n=553)	106.2 (8.3)	0.67 (-0.29, 1.63)	0.173	0.84 (-0.12, 1.80)	0.087
Intervention two					
Placebo (n=564)	106.0 (8.1)	Reference			
Praziquantel (n=555)	105.8 (8.4)	-0.20 (-1.16, 0.77)	0.686		
Level 2: Factors in childhood					
Birth weight (kg) (n=932)		0.73 (-0.33, 1.80)	0.178	0.18 (-0.93, 1.29)	0.751
Sex					
Male (n=583)	105.9 (7.5)	Reference		Reference	
Female (n=536)	105.9 (9.0)	-0.02 (-0.98, 0.95)	0.971	0.12 (-1.18, 0.94)	0.819
Sickle cell trait					
HbAA (n=661)	106.0 (8.4)	Reference			
HbAS (n=141)	105.8 (7.9)	-0.28 (-1.79, 1.23)	0.717		
Season of birth					
Dry (n=651)	106.1 (8.1)	Reference			
Wet (n=468)	105.5 (8.3)	-0.56 (-1.54, 0.42)	0.261		
Place of delivery					
Entebbe Hospital (n=824)	105.8 (8.2)	Reference		Reference	
Home (n=120)	104.9 (8.6)	-0.86 (-2.43, 0.71)		-0.37 (-3.71, 2.96)	
Others (n=174)	106.8 (8.0)	0.95 (-0.39, 2.29)	0.166	0.90 (-0.87, 2.68)	0.582
Feeding status (6 weeks of age)					

Exclusively breast fed (n=748)	106.1 (8.2)	Reference			
Mixed fed (n=344)	105.4 (8.4)	-0.70 (-1.75, 0.35)			
Weaned (n=14)	105.8 (7.1)	-0.28 (-4.63, 4.08)	0.430		
Intervention three					
Placebo (n=553)	105.5 (8.4)	Reference			
Albendazole (n=554)	106.1 (8.0)	0.61 (-0.36, 1.58)	0.218		
HIV status					
Unexposed (n=1001)	106.0 (8.3)	Reference		Reference	
Exposed not infected (n=100)	105.2 (7.3)	-0.83 (-2.52, 0.86)		-0.29 (-2.15, 1.57)	
Infected (n=18)	102.7 (6.1)	-3.34 (-7.17, 0.49)	0.156	-3.85 (-7.81, 0.12)	0.157
Malaria infection below 5 years of age					
Clinical or asymptomatic[a]					
None (n=456)	106.6 (8.0)	Reference		**Reference**	
Yes (n=663)	105.3 (8.3)	-1.31 (-2.29, -0.33)	0.009	**-1.24 (-2.32, -0.17)**	**0.023**
Clinical malaria[a]					
None (n=474)	106.6 (8.0)	Reference		**Reference**	
Yes (n=645)	105.4 (8.3)	-1.19 (-2.17, -0.22)	0.016	**-1.08 (-2.15, -0.02)**	**0.045**
Episodes of clinical malaria[a]					
None (n=474)	106.6 (8.0)	Reference		Reference	
1-2 (n=382)	105.4 (8.4)	-1.13 (-2.24, -0.03)		-1.11 (-2.32, 0.11)	
≥ 3 (n=263)	105.3 (8.2)	-1.28 (-2.52, -0.04)	0.026 [trend]	-1.05 (-2.41, 0.31)	0.133
Asymptomatic malaria[a]					
None (n=983)	106.1 (8.2)	Reference		**Reference**	
Yes (n=124)	103.7 (8.0)	-2.41 (-3.94, -0.88)	0.002	**-1.95 (-3.70, -0.20)**	**0.028**
Schistosomiasis					
Uninfected (n=1076)	105.9 (8.2)	Reference			
Infected (n=33)	104.8 (7.9)	-1.09 (-3.94, 1.76)	0.452		
Ascaris					
Uninfected (n=1052)	105.9 (8.3)	Reference			
Infected (n=57)	105.3 (7.3)	-0.62 (-2.82, 1.57)	0.576		
Hookworm					
Uninfected (n=1085)	105.9 (8.2)	Reference			
Infected (n=24)	103.8 (8.9)	-2.06 (-5.38, 1.27)	0.225		
Trichuris					
Uninfected (n=997)	105.9 (8.2)	Reference			
Infected (n=112)	105.6 (8.6)	-0.28 (-1.89, 1.33)	0.731		
Microfilaria					
Uninfected (n=1102)	105.8 (8.2)	Reference			
Infected (n=8)	109.4 (8.9)	3.58 (-2.13, 9.28)	0.219		
Level 3: Factors in adolescence					
Age (years)		2.12 (1.17, 3.08)	<0.001	**1.35 (0.32, 2.39)**	**0.009**
Body mass index (kg/m^2)		1.27 (1.02, 1.51)	<0.001	**0.78 (0.42, 1.14)**	**<0.001**
Waist circumference		0.46 (0.36, 0.55)	<0.001	**0.21 (0.08, 0.35)**	**0.002**
Family history					
High blood pressure					
No (n=1000)	105.7 (8.1)	Reference		**Reference**	
Yes (n=105)	107.6 (8.3)	1.88 (0.24, 3.52)	0.025	**1.84 (0.12, 3.56)**	**0.034**
Diabetes					
No (n=927)	105.8 (8.0)	Reference			
Yes (n=186)	106.4 (9.2)	0.69 (-0.61, 1.99)	0.296		
Body composition analysis [c]					
Fat mass index[b] (kg/m^2) (n=176)		3.27 (2.29, 4.24)	<0.001	1.50 (-0.38, 3.38)	0.089
Fat free mass index[b] (kg/m^2) (n=176)		1.54 (0.65, 2.43)	0.001	-0.86 (-2.25, 0.54)	0.188
Total body water index[b] (kg/m^2) (n=176)		4.20 (2.97, 5.42)	<0.001	2.51 (-0.24, 5.27)	0.052
Adding salt to food					
No (n=20)	106.2 (7.3)	0.36 (-3.28, 4.00)			
Yes (n=1086)	105.9 (8.2)	Reference	0.846		
Days a fruit is eaten/week					
0-2 (n=543)	106.3 (8.0)	Reference		Reference	
3-7 (n=541)	105.5 (8.5)	-0.83 (-1.82, 0.15)	0.098	-0.83 (-1.84, 0.19)	0.106
Days vegetables eaten/week					
0-2 (n=461)	106.4 (8.2)	Reference		**Reference**	
3-7 (n=635)	105.5 (8.3)	-0.94 (-1.93, 0.05)	0.063	**-1.13 (-2.15, -0.10)**	**0.029**
Days animal-protein eaten/week					

0-2 (n=726)	105.4 (7.8)	Reference		Reference	
3-7 (n=374)	106.6 (8.8)	1.17 (0.16, 2.19)	0.024	0.99 (-0.06, 2.04)	0.062
Days sugared drinks taken/week					
None (n=427)	105.4 (8.1)	Reference		Reference	
1-3 (n=492)	105.9 (8.0)	0.54 (-0.53, 1.61)		-0.05 (-1.14, 1.05)	
4-7 (n=174)	107.2 (9.1)	1.81 (0.36, 3.26)	0.051	0.96 (-0.53, 2.44)	0.358
Days a fruit is eaten/week		-0.05 (-0.27, 0.18)	0.687		
Days vegetables eaten/week		-0.18 (-0.39, 0.03)	0.085	-0.19 (-0.40, 0.03)	0.081
Days animal-protein eaten/week		0.21 (-0.07, 0.50)	0.138	0.10 (-0.20, 0.39)	0.502
Days starchy foods eaten/week		0.14 (-0.45, 0.73)	0.636		
Days sugared drinks taken/week		0.23 (0.00, 0.46)	0.049	0.11 (-0.12, 0.35)	0.325
Breast development (girls only)[b]					
Pre-pubertal (n=178)	103.9 (7.8)	Reference		Reference	
Pubertal (n=97)	108.0 (10.5)	4.07 (1.87, 6.26)	<0.001	1.17 (-1.26, 3.59)	0.318
Pubic hair development[a]					
Pre-pubertal (n=441)	104.7 (7.4)	Reference		Reference	
Pubertal (n=170)	106.5 (9.3)	1.83 (0.42, 3.24)	0.011	0.51 (-0.96, 1.98)	0.486
Snoring					
No (n=932)	105.8 (8.2)	Reference			
Yes (n=163)	106.3 (8.2)	0.53 (-0.83, 1.90)	0.444		
Duration of night sleep					
< 9 hrs (n=306)	106.1 (8.0)	Reference			
9 hrs (n=382)	105.8 (8.8)	-0.28 (-1.51, 0.96)			
> 9hrs (n=405)	105.7 (7.7)	-0.39 (-1.61, 0.83)	0.818		
Smoking in household					
No (n=962)	106.0 (8.3)	Reference		Reference	
Yes (n=147)	104.9 (7.5)	-1.03 (-2.46, 0.40)	0.157	-0.65 (-2.10, 0.80)	0.372
Type of school					
Day (n=117)	105.7 (7.9)	Reference		Reference	
Boarding school (n=719)	107.5 (10.3)	1.76 (0.19, 3.34)	0.038	0.28 (-1.38, 1.95)	0.733
Physical education at school					
No (n=385)	105.5 (8.5)	Reference			
Yes (n=719)	106.0 (8.1)	0.48 (-0.54, 1.50)	0.360		
Infections at BP measurement					
Asymptomatic malaria					
Uninfected (n=1067)	106.0 (8.2)	Reference		Reference	
Infected (n=22)	103.1 (9.3)	-2.85 (-6.31, 0.61)	0.106	-1.50 (-5.02, 2.02)	0.397
Schistosomiasis					
Uninfected (n=964)	105.9 (8.3)	Reference			
Infected (n=112)	105.7 (8.4)	-0.25 (1.88, 1.38)	0.764		
Hookworm					
Uninfected (n=1066)	105.9 (8.3)	Reference			
Infected (n=10)	103.8 (10.0)	-2.10 (-7.27, 3.07)	0.425		
Ascaris					
Uninfected (n=1073)	105.9 (8.3)	Reference		Reference	
Infected (n=3)	98.7 (1.6)	-7.34 (-16.65, 2.17)	0.132	-7.04 (-15.97, 1.88)	0.117
Trichuris					
Uninfected (n=1036)	105.8 (8.3)	Reference		**Reference**	
Infected (n=40)	107.9 (8.3)	2.16 (-0.46, 4.78)	0.106	**3.48 (0.79, 6.18)**	**0.010**

Model building followed the hierarchical approach, adding factors sequentially at three levels starting with the distal factors (level 1). Factors at the same level were added to the model at the same time and considered confounders for each other and for proximal factors. A p-value <0.20 was used for considering factor for inclusion and maintaining factors in the model.

Adjusted β with 95% CI excluding 0 in bold.

β; linear regression coefficient: mean difference in blood pressure (BP) measured in mmHg.

[a] Not included in the model together but each was adjusted for all other model variables.

[b] Not included in multivariable model building for other exposures because of large proportion of missing information but each was adjusted for variables in the final model building.

[c] Not adjusted for body mass index because body mass index is on the causal pathway.

Table 2: Factors investigated for association with diastolic blood pressure among adolescents from the Entebbe Mother and Baby Study (N=1119)

Level 1: Maternal factors	Mean BP (SD)	Crude β (95% CI)	P-value	Adjusted β (95% CI)	P-value
Age (years)		0.08 (-0.00, 0.15)	0.058	0.05 (-0.03, 0.13)	0.247
Household SES (n=1104)		0.22 (-0.13, 0.56)	0.225		
Parity		0.08 (-0.16, 0.32)	0.530		
Body mass index (n=1110)		0.16 (0.03, 0.29)	0.014	**0.14(0.01, 0.27)**	**0.030**
Education status					
None (n=28)	65.1 (9.3)	0.44 (-2.32, 3.19)		**0.08 (-2.71, 2.89)**	
Primary (n=542)	64.6 (6.7)	Reference		**Reference**	
Senior (n=438)	65.5 (7.5)	0.92 (0.01, 1.84)		**1.00 (0.07, 1.92)**	
Tertiary (n=109)	66.8 (8.0)	2.14 (0.65, 3.64)	0.023	**2.08 (0.57, 3.59)**	**0.022**
Marital status					
Single (n=116)	64.2 (6.4)	-1.19 (-2.59, 0.21)		-1.26 (-2.69, 0.16)	
Married/cohabiting (n=967)	65.4 (7.4)	Reference		Reference	
Separated/widowed (n=35)	63.5 (6.0)	-1.91 (-4.36, 0.54)	0.089	-1.91 (-4.38, 0.54)	0.075
Area of residence					
Urban (n=770)	65.3 (7.5)	Reference			
Rural (n=336)	64.9 (6.8)	0.49 (-1.42, 0.44)	0.302		
Alcohol use					
No (n=775)	65.3 (7.5)	Reference			
Yes (n=343)	65.0 (6.6)	-0.34 (-1.26, 0.59)	0.477		
Infections					
HIV					
Uninfected (n=1002)	65.2 (7.3)	Reference			
Infected (n=117)	64.9 (6.5)	-0.35 (-1.74, 1.05)	0.626		
Asymptomatic malaria					
Uninfected (n=991)	65.2 (7.4)	Reference			
Infected (n=109)	64.9 (6.6)	-0.29 (-1.73, 1.15)	0.695		
Schistosomiasis					
Uninfected (n=908)	65.2 (7.1)	Reference			
Infected (n=204)	65.5 (7.7)	0.31 (-0.79, 1.41)	0.579		
Hookworm					
Uninfected (n=662)	65.1 (7.1)	Reference			
Infected (n=450)	65.4 (7.4)	0.27 (-0.60, 1.14)	0.539		
Ascaris					
Uninfected (n=1084)	65.3 (7.3)	Reference			
Infected (n=28)	65.1 (5.5)	-0.18 (-2.90, 2.54)	0.896		
Intervention one					
Placebo (n=566)	65.0 (6.9)	Reference			
Albendazole (n=553)	65.4 (7.7)	0.39 (-0.46, 1.24)	0.366		
Intervention two					
Placebo (n=564)	65.4 (7.3)	Reference			
Praziquantel (n=555)	65.0 (7.2)	-0.44 (-1.29, 0.42)	0.315		
Level 2: Factors in childhood					
Birth weight (kg) (n=932)		0.66 (-0.27, 1.59)	0.164	0.57 (-0.40, 1.53)	0.246
Sex					
Male (n=583)	64.9 (7.2)	Reference		Reference	
Female (n=536)	65.5 (7.4)	0.64 (-0.21, 1.49)	0.141	0.49 (-0.43, 1.42)	0.294
Sickle cell trait					
HbAA (n=661)	65.4 (7.1)	Reference			
HbAS (n=141)	65.5 (7.4)	0.15 (-1.16, 1.46)	0.825		
Season of birth					
Dry (n=651)	65.5 (7.3)	Reference		Reference	
Wet (n=468)	64.7 (7.2)	-0.79 (-1.65, 0.07)	0.073	0.59 (-1.52, 0.35)	0.214
Place of delivery					
Entebbe Hospital (n=824)	65.1 (7.1)	Reference			
Home (n=120)	65.4 (8.5)	0.36 (-1.03, 1.76)			
Others (n=174)	65.7 (7.3)	0.61 (-0.58, 1.80)	0.564		
Feeding status (6 week of age)					
Exclusive Breastfed (n=748)	65.4 (7.4)	Reference			
Mixed fed (n=344)	64.7 (7.0)	-0.63 (-1.56, 0.30)			
Weaned (n=14)	67.1 (4.4)	1.78 (-2.07, 5.63)	0.251		
Intrvention three					

Placebo (n=553)	64.9 (7.0)	Reference		Reference	
Albendazole (n=554)	65.5 (7.5)	0.62 (-0.24, 1.47)	0.156	0.56 (-0.37, 1.48)	0.233
HIV status					
Unexposed (n=1001)	65.2 (7.3)	Reference			
Exposed not infected (n=100)	65.1 (6.7)	-0.12 (-1.62, 1.37)			
Infected (n=18)	63.5 (5.1)	-1.71 (-5.10, 1.68)	0.609		
Malaria infection below 5 years of age					
Clinical or asymptomatic malaria[a]					
No (n=447)	65.9(7.1)	Reference		**Reference**	
Yes (n=663)	64.6 (7.3)	-1.28 (-2.14, -0.41)	0.004	**-1.47 (-2.41, -0.53)**	**0.002**
Clinical malaria[a]					
None (n=474)	66.0 (7.2)	Reference		**Reference**	
Yes (n=645)	64.6 (7.3)	-1.38 (-2.24, -0.51)	0.002	**-1.33 (-2.26, -0.39)**	**0.005**
Episodes of clinical malaria[a]					
None (n=474)	65.9 (7.2)	Reference		**Reference**	
1-2 (n=382)	64.5 (7.3)	-1.45 (-2.42, -0.47)		**-1.53 (-2.59, -0.46)**	
≥ 3 (n=263)	64.9 (7.4)	-1.02 (-2.12, 0.07)	0.011	**-1.03 (-2.22, 0.16)**	**0.015**
Asymptomatic malaria[a]					
None (n=983)	64.5 (7.3)	Reference		Reference	
Yes (n=124)	64.9 (7.4)	-1.45 (-2.80, -0.10)	0.035	-1.35 (-2.89, 0.18)	0.082
Schistosomiasis					
Uninfected (n=1076)	65.2 (7.3)	Reference			
Infected (n=33)	64.5 (5.8)	0.67 (-3.18, 1.84)	0.602		
Ascaris					
Uninfected (n=1052)	65.2 (7.3)	Reference			
Infected (n=57)	64.5 (7.1)	-0.75 (-2.68, 1.18)	0.445		
Hookworm					
Uninfected (n=1085)	65.2 (7.3)	Reference		Reference	
Infected (n=24)	62.9 (5.8)	-2.29 (-5.22, 0.64)	0.125	-1.79 (-4.93, 1.35)	0.261
Trichuris					
Uninfected (n=997)	65.1 (7.2)	Reference			
Infected (n=112)	65.8 (7.7)	0.67 (-0.74, 2.09)	0.353		
Microfilaria					
Uninfected (n=1102)	65.1 (7.2)	Reference			
Infected (n=8)	67.3 (3.3)	2.12 (-2.91, 7.14)	0.409		
Level 3: Factors in adolescence					
Age (years)		1.85(1.00, 2.70)	<0.001	**1.53 (0.63, 2.43)**	**<0.001**
Body mass index (kg/m^2)		0.28 (0.20, 0.36)	<0.001	**0.74 (0.42, 1.05)**	**<0.001**
Waist circumference (cm)		0.88 (0.66, 1.10)	<0.001	0.07 (-0.05, 0.18)	0.279
Family history					
High blood pressure					
No (n=1000)	65.0 (7.2)	Reference		**Reference**	
Yes (n=105)	66.7 (7.6)	1.65 (0.19, 3.12)	0.027	**1.57 (0.08, 3.06)**	**0.037**
Diabetes					
No (n=927)	65.2 (7.2)	Reference			
Yes (n=186)	65.5 (7.8)	0.35 (-0.80, 1.49)	0.553		
Body composition analysis[c]					
Fat mass index[b] (kg/m^2) (n=176)		1.75 (0.83, 2.69)	<0.001	0.87 (-0.73, 2.47)	0.255
Fat-free mass index[b] (kg/m^2) (n=176)		1.19 (0.40, 1.98)	0.003	0.28 (-0.90, 1.45)	0.622
Total body water index[b] (kg/m^2) (n=176)		2.13 (0.95, 3.30)	<0.001	1.51 (-0.86, 3.88)	0.180
Adding salt to food					
No (n=20)	67.4 (6.1)	2.19 (-1.04, 5.41)		2.72 (-0.39, 5.82)	
Yes (n=1086)	65.2 (7.3)	Reference	0.184	Reference	0.083
Days a fruit is eaten/week					
0-2 (n=543)	65.7 (7.1)	Reference		**Reference**	
3-7 (n=541)	64.7 (7.5)	-0.98 (-1.85, -0.11)	0.028	**-0.96 (-1.83, -0.10)**	**0.027**
Days vegetables eaten/week					
0-2 (n=461)	65.4 (7.1)	Reference			
3-7 (n=635)	65.1 (7.5)	-0.27 (-1.15, 0.60)	0.540		
Days animal-protein eaten/week					
0-2 (n=726)	65.1 (6.9)	Reference			
3-7 (n=374)	65.4 (8.0)	0.30 (-0.61, 1.20)	0.523		
Days sugared drinks taken/week					
None (n=427)	65.0 (7.1)	Reference		Reference	
1-3 (n=492)	65.2 (7.4)	0.25 (-0.70, 1.20)		0.12 (-0.84, 1.08)	

4-7 (n=174)	66.0 (7.5)	1.06 (-0.23, 2.35)	0.271	0.54 (-0.75, 1.83)	0.707
Days a fruit is eaten/week					
Days vegetables eaten/week		0.02 (-0.16, 0.1)	0.800		
Days animal-protein eaten/week		0.14 (-0.11, 0.39)	0.284		
Days starchy foods eaten/week		0.03 (-0.50, 0.55)	0.924		
Days sugared drinks taken/week		0.20 (0.00, 0.41)	0.048		
Breast development (girls only)[b]					
Pre-pubertal (n=178)	64.1 (6.1)	Reference		Reference	
Pubertal (n=97)	67.2 (7.9)	3.07 (1.38, 4.76)	<0.001	0.98 (-0.88, 2.84)	0.281
Pubic hair development[b]					
Pre-pubertal (n=441)	64.1 (6.6)	Reference		Reference	
Pubertal (n=170)	66.1 (7.6)	2.04 (0.82, 3.26)	0.001	0.68 (-0.62, 1.99)	0.293
Snoring					
No (n=932)	65.1 (7.2)	Reference			
Yes (n=163)	65.6 (7.8)	0.44 (-0.78, 1.66)	0.477		
Duration of night sleep					
< 9 hrs (n=306)	65.8 (7.6)	Reference		Reference	
9 hrs (n=382)	64.8 (7.1)	-1.03 (-2.11, 0.06)		-0.92 (-2.02, 0.18)	
> 9hrs (n=405)	65.2 (7.2)	-0.79(-1.86, 0.28)	0.160	-0.67 (-1.76, 0.43)	0.240
Smoking in household					
Non (n=962)	65.2 (7.3)	Reference			
Yes (n=147)	65.0 (6.8)	-0.21 (-1.46, 1.06)	0.745		
Type of school					
Day (n=117)	65.1 (7.2)	Reference		Reference	
Boarding school (n=719)	66.2 (7.8)	1.13 (-0.26, 2.52)	0.112	-0.24 (-1.67, 1.20)	0.737
Physical education at school					
No (n=385)	65.0 (6.9)	Reference			
Yes (n=719)	65.3 (7.5)	0.32 (-0.58, 1.22)	0.482		
Infections at BP measurement					
Asymptomatic malaria					
Uninfected (n=1067)	65.3 (7.3)	Reference			
Infected (n=22)	64.0 (5.5)	-1.31 (-4.36, 1.75)	0.401		
Schistosomiasis					
Uninfected (n=964)	65.2 (7.4)	Reference			
Infected (n=112)	65.0 (5.8)	-0.19 (-1.62, 1.24)	0.791		
Hookworm					
Uninfected (n=1066)	65.2 (7.3)	Reference			
Infected (n=10)	64.0 (5.9)	-1.25 (-5.80, 3.30)	0.590		
Ascaris					
Uninfected (n=1073)	65.2 (7.3)	Reference			
Infected (n=3)	62.3 (4.3)	-2.86 (-11.14, 5.42)	0.498		
Trichuris					
Uninfected (n=1036)	65.1 (7.2)	Reference			
Infected (n=40)	66.4 (9.4)	1.23 (-1.07, 3.54)	0.294		

Model building followed the hierarchical approach, adding factors sequentially at three levels starting with the distal factors (level 1). Factors at the same level were added to the model at the same time and considered confounders for each other and for proximal factors. A p-value <0.20 was used for considering factor for inclusion and maintaining factors in the model.

Adjusted β for which 95% CI exclude 0 are highlighted in bold.

β; linear regression coefficient: mean difference in blood pressure (BP) measured in mmHg.

[a] Not included in the model together but each was adjusted for all other variables in the model.

[b] Not included in multivariable model building for other exposures because of large proportion of missing information; but each was adjusted for variables in the final model building.

[c] Not adjusted for body mass index because body mass index is on the causal pathway.

Prevalence of high blood pressure

Using day-one BP readings, the prevalence of pre-hypertension and high BP was 63 (10.8%) and 42 (7.2%), respectively among males, and 54 (10.1%) and 52 (9.7%), respectively among females. After extra measurements on the second and third visits and taking loss to follow-up into account, pre-hypertension prevalence was estimated as 2.2% in males and 0.7% in females; high BP prevalence was 0.4% in males and 1.8% in females.

Risk factors for high blood pressure

Tables 1 and 2 show the relationship between examined characteristics and BP (systolic or diastolic) in adolescents. Maternal factors crudely positively associated with adolescent systolic BP were gestational BMI and education status; both remained associated with systolic BP after adjustment for other maternal factors. The trial interventions during pregnancy (albendazole and praziquantel) and early childhood (albendazole) had no effect on systolic or diastolic BP.

Characteristics at the time of BP measurement showing a crude positive association with systolic BP were age, BMI, waist circumference, family history of high BP, body composition variables, and puberty stage covariates. In multivariable analysis, systolic BP increased, on average, by 1.35mmHg, 95% CI (0.32, 2.39) for each one-year increase in adolescents' age; by 0.78mmHg (0.42, 1.14) per unit increase in BMI; and by 0.21mmHg (0.08, 0.35) per centimetre increase in waist circumference. Family history of high BP remained associated with increased SBP, β= 1.84 (0.12, 3.56) after adjustment for maternal and childhood factors. Body composition and puberty stage covariates were no longer associated with systolic BP on adjusting for adolescents' age, BMI and waist circumference.

Lifestyle factors crudely associated with systolic BP were increased animal protein consumption, and the type of school attended, with some evidence of association for fruit, vegetables and sugared drinks consumption. After adjusting for confounders, systolic BP reduced, on average, by 1.13mmHg (-2.15, -0.10) among adolescents who consumed vegetables for 3-7 days/week (versus 0-2 days/week).

Current infection with *Trichuris* was positively associated with systolic BP after adjusting for confounders (β=3.48mmHg (0.79, 6.18)). systolic BP dropped by 1.24mmHg (-2.32, -0.17) among adolescents who had malaria in childhood compared to those who had not. Both clinical and asymptomatic malaria were independently associated with lower BP in

multivariable analysis. Weight and height at 10 and 11 years of age were reduced among adolescents with childhood clinical and or asymptomatic malaria (Supplementary Table 1). Compared to those with no asymptomatic malaria, having asymptomatic malaria in childhood was associated with, on average, a 3.2 cm reduction in height, 95% CI (-4.5, -2.0) and a 2.1 kg reduction in weight, 95% CI (-3.0, -1.9). The effect of childhood malaria on adolescent BP was weaker on adjusting for adolescent BMI (Supplementary Table 2).

Genetic data were available for 802 (72%) participants of whom 141 (18%) had sickle-cell trait (HbAS) and 661 (82%) normal haemoglobin (HbAA). Sickle-cell trait was not associated with systolic BP (β=-0.28mmHg (-1.79, 1.23)), even after adjusting for age and sex. HbAS was inversely associated with malaria (Supplementary Table 3): in those with HbAA, 63% had clinical or asymptomatic malaria up to 5 years compared to 51% with HbAS (p-value =0.008).

Findings for diastolic BP were broadly similar to those for systolic BP, with the exceptions that higher fruit rather than vegetable consumption was associated with lower diastolic BP, and there was no association with waist circumference or *Trichuris* infection. No associations were observed between adolescent BP and any of the other factors considered in this population (Tables 1 and 2).

Discussion

Persistent high BP and pre-hypertension were unusual in early adolescence in this setting. Maternal gestational BMI and education status at enrolment, participant's family history of hypertension, and age and BMI at BP measurement were positively associated with both systolic BP and diastolic BP. Malaria parasitaemia in childhood, and increased vegetable and fruit consumption were inversely associated with systolic BP and diastolic BP, respectively. Concurrent *Trichuris* infection was positively associated with systolic BP but not with diastolic BP. There were no effects of anti-helminth trial interventions (in pregnancy or childhood) on adolescent BP and no associations between prior helminth infection (in pregnancy or childhood) and adolescent BP.

Our findings are consistent with several earlier studies [25, 26]. We have shown that consuming vegetables and fruits for 3-7 days/week was associated with lower systolic BP and diastolic BP, respectively. Our results support findings from a cross-sectional study that consuming fruits and vegetables (> 400g per day) lowers systolic BP and diastolic BP in adults [26]. We have shown a positive association of BP with maternal gestational BMI, and adolescent BMI and waist circumference at the time of

BP measurement, consistent with earlier studies [13].

Malaria parasitaemia in childhood was associated with lower BP in early adolescence, consistent with findings from a cross-sectional study among 5-18-year-old Ugandan students, which reported that current asymptomatic malaria was associated with lower BP [25]. Our study was under-powered to detect the effect of current parasitaemia on BP, with only 22 (2.1%) adolescents had parasitaemic at the time of BP measurement.

Sub-microscopic malaria was most likely misclassified as negative in this population, since in malaria-endemic areas, asymptomatic malaria often presents as sub-microscopic in individuals with past malaria infection [27]. We found no association between sickle-cell trait and adolescent BP; contrary to the hypothesis advanced by Etyang, who used sickle-cell trait as an instrumental variable in a Mendelian randomisation study [28]. In the predominantly adult populations from Kenya, sickle-cell trait (linked with partial protection against malaria) was associated with lower BP in Kilifi (currently a low-moderate but historically a high malaria transmission area) compared to Nairobi (no malaria transmission) [29]. The differences in malaria exposure intensity and participant age distribution between our study and the Kenyan study could explain our contrasting results.

Similar to earlier studies [30, 31], childhood malaria was associated with reductions in both weight and height, and some of the inverse association seen in this study may be explained by this mechanism, or by confounding by unmeasured factors. The escalating burden of high BP has coincided with the declining malaria burden on the African continent [2, 32, 33]. This could be explained by the epidemiological transition process on continent, or the effect could be more direct; the mechanisms remain to be elucidated.

Current but not previous infection with *Trichuris* (a type of soil transmitted helminth, commonly known as whipworm) was associated with increased systolic BP in early adolescence. To our knowledge, no study has previously reported such an association. This may reflect short-term effects (probably arterial stiffness from inflammatory reaction) or it could be a spurious finding due to the many exposures included in the analysis. The effect of current *Trichuris* infection on BP is likely not mediated through increasing BMI (weight or height); there was no difference in these measures between adolescents with and without current *Trichuris* infection.

Unlike previous studies [34], we found no association between BP and salt intake. The lack of evidence for this relationship in our study could be due to measurement error from self-report, or the fact that nearly everyone added salt to cooked food. Measuring sodium in a 24-hour urine sample or in commonly consumed local foods would provide a more accurate reflection of daily intake. Physical activity was not associated with lower BP, contrary to earlier literature [35]; sedentary lifestyles are still fairly uncommon in this population.

Previous studies have linked hypertension to socioeconomic determinants (socioeconomic status (SES), education, income, urbanisation) [12, 36]. Our study is consistent with a Uganda study in adults which showed that BP was not associated with urban residence [37] but contrary to studies linking increased BP with low SES [36] and urbanisation [12]. We have shown that higher maternal education was associated with increased BP in adolescents, whereas other studies, predominantly from high-income countries, report an inverse association [36]. Although low SES and education is associated with hypertension in the developed world [36], the relationship may be inverse in less developed countries [38]. In these settings, offspring from more highly educated households are more likely to have sedentary lifestyles and unhealthy dietary practices, and to be obese, compared to offspring from less-educated households.

Strengths of this study included its longitudinal design with prospectively collected data reducing recall and reporter bias, the use of robust BP procedures and the measurement of BP on up to two extra occasions in those with BP $\geq 95^{th}$ percentile at the initial visit, to avoid overestimation of high BP. It is unlikely that white-coat phenomenon was an issue as participants regularly attend this clinic for scheduled and/or illness visits. The use of digital machines reduced differences in BP reading between operators which can occur with auscultation.

Study limitations include the possibility of residual confounding by unmeasured factors (such as Glomerular filtration rate (GFR). The GFR could not be estimated as creatinine was only measured for a subgroup of the participants. The use of digital BP machines may overestimate BP; however, digital devices used in this study were calibrated twice annually. A large number of statistical tests were undertaken; thus, some findings may be due to multiplicity. However, it is reassuring that most findings are consistent with previous literature, albeit from different settings. Not inviting all adolescents (those with pre-hypertension or normal BP on day-one) for up to two extra BP measurements might have resulted in an underestimation in the overall prevalence of pre-hypertension and

hypertension. We modelled BP as a continuous outcome, since analysing high or pre-hypertensive BP versus normal BP as a binary outcome (or outcomes) would be underpowered, consequently our findings may not necessarily reflect associations with hypertensive disease.

In summary, routine BP screening which is seldom conducted for adolescents at health care visits remains vital in the control and prevention of CVDs later in life. Similar life-course factors to those observed in high-income settings (such as adiposity and diet) affect both systolic BP and diastolic BP among African adolescents. Interventions during pregnancy, childhood and early adolescence could be vital in the control and prevention of later high BP. Multiple intervention strategies initiated during pregnancy and the early postnatal period and continued across a lifetime could be fundamental in the control of adulthood hypertension and CVDs.

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Conflict of interest

The authors declare that they have no conflict of interest

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References

1. Donnison, C.P., Pressure in the African Native: its bearing upon the aetiology of hyperpiesia and arteriosclerosis. The Lancet, 1929. **213**(5497): p. 6-7.
2. Dalal, S., et al., Non-communicable diseases in sub-Saharan Africa: what we know now. Int J Epidemiol, 2011. **40**(4): p. 885-901.
3. Lopez, A.D., et al., Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet, 2006. **367**(9524): p. 1747-57.
4. Kagura, J., et al., Blood pressure tracking in urban black South African children: birth to twenty cohort. BMC Pediatr, 2015. **15**: p. 78.
5. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics, 2004. **114**(2 Suppl 4th Report): p. 555-76.
6. Hardy, R., D.A. Lawlor, and D. Kuh, A life course approach to cardiovascular aging. Future Cardiol, 2015. **11**(1): p. 101-13.
7. Din-Dzietham, R., et al., High blood pressure trends in children and adolescents in national surveys, 1963 to 2002. Circulation, 2007. **116**(13): p. 1488-96.
8. Noubiap, J.J., et al., Prevalence of elevated blood pressure in children and adolescents in Africa: a systematic review and meta-analysis. The Lancet Public Health, 2017. **2**(8): p. e375-e386.
9. Gill, D.G., et al., Analysis of 100 children with severe and persistent hypertension. Arch Dis Child, 1976. **51**(12): p. 951-6.
10. Falkner, B. and S.R. Daniels, Summary of the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents. Hypertension, 2004. **44**(4): p. 387-8.
11. Hansen, M.L., P.W. Gunn, and D.C. Kaelber, Underdiagnosis of hypertension in children and adolescents. JAMA, 2007. **298**(8): p. 874-9.
12. Addo, J., L. Smeeth, and D.A. Leon, Hypertension in sub-saharan Africa: a systematic review. Hypertension, 2007. **50**(6): p. 1012-8.

13. Jobe, M., et al., High blood pressure and associated risk factors as indicator of preclinical hypertension in rural West Africa: A focus on children and adolescents in The Gambia. Medicine (Baltimore), 2017. **96**(13): p. e6170.

14. Afrifa-Anane, E., et al., The association of physical activity, body mass index and the blood pressure levels among urban poor youth in Accra, Ghana. BMC Public Health, 2015. **15**: p. 269.

15. Elliott, A.M., et al., The impact of helminths on the response to immunization and on the incidence of infection and disease in childhood in Uganda: design of a randomized, double-blind, placebo-controlled, factorial trial of deworming interventions delivered in pregnancy and early childhood [ISRCTN32849447]. Clin Trials, 2007. **4**(1): p. 42-57.

16. Ndibazza, J., et al., Associations between maternal helminth and malaria infections in pregnancy and clinical malaria in the offspring: a birth cohort in entebbe, Uganda. J Infect Dis, 2013. **208**(12): p. 2007-16.

17. Lule, S.A., et al., Are birthweight and postnatal weight gain in childhood associated with blood pressure in early adolescence? Results from a Ugandan birth cohort. Int J Epidemiol, 2018.

18. Marshall, W.A. and J.M. Tanner, Variations in pattern of pubertal changes in girls. Arch Dis Child, 1969. **44**(235): p. 291-303.

19. Gurdasani, D., et al., The African Genome Variation Project shapes medical genetics in Africa. Nature, 2015. **517**(7534): p. 327-32.

20. Katz, N., A. Chaves, and J. Pellegrino, A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo, 1972. **14**(6): p. 397-400.

21. Webb, E.L., et al., Effect of single-dose anthelmintic treatment during pregnancy on an infant's response to immunisation and on susceptibility to infectious diseases in infancy: a randomised, double-blind, placebo-controlled trial. Lancet, 2011. **377**(9759): p. 52-62.

22. Melrose, W.D., et al., An improved Knott's concentration test for the detection of microfilariae. Trans R Soc Trop Med Hyg, 2000. **94**(2): p. 176.

23. Muhangi, L., et al., Associations between mild-to-moderate anaemia in pregnancy and helminth, malaria and HIV infection in Entebbe, Uganda. Trans R Soc Trop Med Hyg, 2007. **101**(9): p. 899-907.

24. Woodburn, P.W., et al., Risk factors for helminth, malaria, and HIV infection in pregnancy in Entebbe, Uganda. PLoS Negl Trop Dis, 2009. **3**(6): p. e473.

25. Kidy, F., et al., Blood pressure in primary school children in Uganda: a cross-sectional survey. BMC Public Health, 2014. **14**: p. 1223.

26. Pienovi, L., et al., [Fruit and vegetable intake, and blood pressure. A population research]. Arch Latinoam Nutr, 2015. **65**(1): p. 21-6.

27. Lin, J.T., D.L. Saunders, and S.R. Meshnick, The role of submicroscopic parasitemia in malaria transmission: what is the evidence? Trends Parasitol, 2014. **30**(4): p. 183-90.

28. Etyang, A.O.S., L.;Cruickshank, J. K.;Scott, J. A. G., New hypotheses in clinical medicine: the malaria-high blood pressure hypothesis. Circulation Research, 2016. **119**(1): p. 36-40.

29. Etyang, A.O., Determining the Causal Role of Malaria in Elevating Blood Pressure and Pulse Wave Velocity in Kenyan Adolescents and Adults, in Department of Infectious Disease Epidemiology. 2017, London School of Hygiene & Tropical Medicine.

30. ter Kuile, F.O., et al., Impact of permethrin-treated bed nets on malaria, anemia, and growth in infants in an area of intense perennial malaria transmission in western Kenya. Am J Trop Med Hyg, 2003. **68**(4 Suppl): p. 68-77.

31. Bradley-Moore, A.M., et al., Malaria chemoprophylaxis with chloroquine in young Nigerian children. III. Its effect on nutrition. Ann Trop Med Parasitol, 1985. **79**(6): p. 575-84.

32. Bhatt, S., et al., The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature, 2015. **526**(7572): p. 207-211.

33. Noor, A.M., et al., The changing risk of Plasmodium falciparum malaria infection in Africa: 2000-10: a spatial and temporal analysis of transmission intensity. Lancet, 2014. **383**(9930): p. 1739-47.

34. Brown, I.J., et al., Salt intakes around the world: implications for public health. Int J Epidemiol, 2009. **38**(3): p. 791-813.

35. Diaz, K.M. and D. Shimbo, Physical activity and the prevention of hypertension. Curr Hypertens Rep, 2013. **15**(6): p. 659-68.

36. Leng, B., et al., Socioeconomic status and hypertension: a meta-analysis. J Hypertens, 2015. **33**(2): p. 221-9.

37. Guwatudde, D., et al., The Epidemiology of Hypertension in Uganda: Findings from the National Non-Communicable Diseases Risk Factor Survey. PLoS One, 2015. **10**(9): p. e0138991.

38. Caballero, B., The global epidemic of obesity: an overview. Epidemiol Rev, 2007. **29**: p. 1-5.

6.3 Supplementary tables

Table S1 Relationship between infection and anthropometry at 10 and 11 years of age

Malaria infection in childhood (≤5 years of age)	**Weight (kg)**		**Height (cm)**	
Clinical or asymptomatic malaria	**Mean (95% CI)**	**p-value**	**Mean (95% CI)**	**p-value**
Uninfected (n=456)	30.2 (29.7, 30.7)		136.7 (136.0, 137.4)	
Infected (663)	29.1 (28.7, 29.4)		135.0 (134.4, 135.5)	
Difference (Uninfected-infected)	1.2 (0.5, 1.8)	<0.001	1.7 (0.9, 2.6)	<0.001
Clinical malaria				
Uninfected (n=474)	30.2 (29.7, 30.7)		136.7 (136.0, 137.3)	
Infected (n=645)	29.1 (28.7, 29.5)		134.9 (134.4, 135.4)	
Difference (Uninfected-infected)	1.1 (0.5, 1.7)	<0.001	1.7 (0.9, 2.5)	<0.001
Asymptomatic malaria				
Uninfected (n=983)	29.8 (29.4, 30.1)		136.0 (135.6, 136.50	
Infected (n=124)	27.7 (26.9, 28.5)		132.8 (131.7, 133.9)	
Difference (Uninfected-infected)	2.1 (1.1, 3.0)	<0.001	3.3 (2.0, 4.5)	<0.001
Current Trichuris infection				
Uninfected (1,0376)	29.5 (29.2, 29.9)		135.7 (135.3, 136.1)	
Infected (n=40)	29.4 (28.2, 30.7)		133.5 (131.6, 135.5)	
Difference (Uninfected-infected)	0.1 (-1.5, 1.8)	0.900	2.2 (0.0, 4.4)	0.049

Table S2. Association between malaria and blood pressure among adolescents from the Entebbe Mother and Baby Study (N=1119)

Systolic BP					
Childhood malaria[1] (≤5 years of age)	**Mean BP (SD)**	**Crude β (95% CI)**	**P-value**	**Adjusted[2] β (95% CI)**	**P-value**
Clinical or asymptomatica					
No (n=456)	106.7 (8.0)	Reference		**Reference**	
Yes (n=663)	105.3 (8.3)	-1.44 (-2.42, -0.46)	0.009	**-1.18 (-2.12, -0.24)**	**<0.001**
Clinical malaria					
No (n=474)	106.6 (8.0)	Reference		**Reference**	
Yes (n=645)	105.4 (8.3)	-1.19 (-2.17, -0.22)	0.016	**-0.97 (-1.91, -0.04)**	**<0.001**
Episodes of clinical malaria					
None (n=474)	106.6 (8.0)	Reference		**Reference**	
1-2 (n=382)	105.4 (8.4)	-1.13 (-2.24, -0.03)		-0.79 (-1.85, 0.28)	
≥3 (n=263)	105.3 (8.2)	-1.28 (-2.52, -0.04)	0.026 [trend]	-1.24 (-2.43, -0.6)	**<0.001**
Asymptomatic malaria					
No (n=983)	106.1 (8.2)	Reference		**Reference**	
Yes (n=124)	103.7 (8.0)	-2.41 (-3.94, -0.88)	0.002	**-1.95 (-3.42, -0.48)**	**<0.001**
Diastolic BP					
Clinical or asymptomatic malaria					
No (n=447)	66.0 (7.2)	Reference		**Reference**	
Yes (n=663)	64.6 (7.3)	-1.38 (-2.24, -0.51)	0.002	**-1.20 (-2.04, -0.35)**	**<0.001**
Clinical malaria[1]					
No (n=474)	65.9 (7.2)	Reference		**Reference**	
Yes (n=645)	64.7 (7.3)	-1.27 (-2.13, -0.41)	0.004	**-1.12 (-1.96, -0.28)**	**<0.001**
Episodes of clinical malaria					
None (n=474)	65.9 (7.2)	Reference		**Reference**	
1-2 (n=382)	64.5 (7.3)	-1.45 (-2.42, -0.47)		**-1.21 (-2.16, -0.25**	
≥3 (n=263)	64.9 (7.4)	-1.02 (-2.12, 0.07)	0.011	**-1.00 (-2.06, 0.07)**	**<0.001**
Asymptomatic malaria					
No (n=983)	65.3 (7.3)	Reference		**Reference**	
Yes (n=124)	63.9 (6.7)	-1.45 (-2.80, -0.10)	0.035	**-1.13(-2.44, 0.19)**	**<0.001**

[1] Childhood malaria is malaria in children aged ≤5 years
[2] Adjusted for current BMI

Table S3 Relationship between sickle-cell trait and malaria infections among Entebbe Mother and Baby Study participants

Malaria infection in childhood (≤5 years of age)	HbAA (n=661)		HbAS (n=141)		
	Frequency	Percentage	Frequency	Percentage	P-value
Clinical or asymptomatic malaria					
No (n=447)	244	36.9	69	48.9	
Yes (n=663)	417	63.1	72	51.1	0.008
Clinical malaria					
None (n=474)	252	38.1	70	49.7	
Yes (n=645)	409	61.9	71	50.4	0.011
Episodes of clinical malaria					
None (n=474)	252	38.1	70	49.7	
1-2 (n=382)	229	34.6	49	34.8	
≥ 3 (n=263)	180	27.2	22	15.6	0.006
Asymptomatic malaria					
None (n=983)	58	88.1	127	90.1	
Yes (n=124)	79	12.0	14	9.9	0.496

Chapter 7: Genetics of blood pressure among Ugandan adolescents

7.1 Introduction

This chapter uses both phenotypic and genotypic data from the EMaBS birth cohort to answer thesis objective 5 which investigates genetic polymorphisms associated with BP among Ugandan adolescents.

7.2 Research paper 4: A genome-wide association and replication study of blood pressure in Ugandan adolescents

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

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SECTION A – Student Details

Student	Abubaker Swaib Lule
Principal Supervisor	Emily Webb
Thesis Title	Investigating the impact of early-life, life-course and genetic factors on blood pressure among young Africans

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

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Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceptualised and designed the study, drafted the study documents (including protocol, standard operating procedures, questionnaires, consent and assent information sheets and forms), conducted the study (including data collection, data

	management and cleaning), performed statistical analysis, interpreted the findings, and revised final manuscript

Student Signature: _____ **Date:** 2/10/2018

Supervisor Signature: Emily Webb **Date:** 2/10/2018.

A genome-wide association and replication study of blood pressure in Ugandan adolescents

Swaib A. Lule[1,2], Alexander J. Mentzer[3,4], Benigna Namara[2], Allan Muwenzi[5], Beatrice Nassanga[2], Helen Akurut[2], Lawrence Lubyayi[2], Josephine Tumusiime[2], Florence Akello[2], Liam Smeeth[1], Alison M. Elliott[1,2], Emily L. Webb[1]

[1] London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

[2] MRC/UVRI & LSHTM Uganda Research Unit, P.O. Box 49, Entebbe, Uganda

[3] Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom

[4] Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, UK

[5] University of Cambridge, Cambridge, United Kingdom

Abstract

Genetic association studies predominantly in Caucasian and Asian populations have identified variants associated with blood pressure (BP). However, in Africa, where high genetic diversity and high BP are prevalent, it is not clear if these or other variants are associated with BP or whether associations are consistent for different age groups. Using data from the Entebbe Mother and Baby Study (EMaBS), we conducted a genome-wide association study (GWAS) of BP and a replication study of single nucleotide polymorphisms (SNPs) found to be associated in previously published GWAS of BP.

Systolic and diastolic BP were measured three times among 10- and 11-year olds in the EMaBS and the means of the last two measurements used. Whole genome genotype data was generated for EMaBS participants using Illumina omni 2.5M arrays and untyped genetic variants or selected variants (for the replication analysis) were imputed. GWAS was conducted using linear mixed model regression analysis to examine variants associated with systolic or diastolic BP. Linear regression analysis was used to assess variants previously associated with systolic or diastolic BP (p-value $<5.0\times10^{-8}$) from published BP GWASs.

14 million variants from 815 adolescents were analysed. No variant reached genome-wide significance (p-value $<5.0\times10^{-8}$) for association with systolic or diastolic BP. The most strongly associated variants were rs181430167, p-value $=6.8\times10^{-7}$ and rs12991132, p-value $=4.0\times10^{-7}$ for systolic and diastolic BP, respectively. Replication analysis was conducted for 330 SNPs previously associated with BP. Respectively, 17 SNPs, 15 SNPs and one SNP were associated with systolic BP, diastolic BP and with both traits (p-value <0.05).

There was no genome-wide evidence of variants influencing BP among Ugandan adolescents, but the few variants showing suggestive associations may be worthy of future investigation. Replication of a small number of previously identified variants suggests that genetic variants associated with BP among African adolescents may overlap somewhat with those already established in previous studies, largely based on adults in Western settings.

Keywords: Blood pressure, genetics, single nucleotide polymorphisms, adolescents, Africa, Uganda

Running title: Blood pressure genetics of African adolescents

Introduction

Genome-wide association studies (GWAS) predominantly from Caucasian and Asian populations have identified several genetic variants/single nucleotide polymorphisms (SNPs) associated with blood pressure (BP) [1-6]. Despite having a high burden of hypertension, and opportunities for improved fine-mapping of causative variants (including higher genetic diversity and lower linkage disequilibrium (LD) [7-10]), African populations are underrepresented in published genetic studies of BP [11]. Among 38 studies that investigated genetic polymorphisms associated with hypertension in Africa-based populations (participant numbers ranging from 65 to 1,939) reviewed in [12], all adopted a candidate gene approach rather than conducting a GWAS. It remains unclear whether variants associated with BP in non-African populations also influence BP among Africans or whether the patterns of genetic susceptibility differ markedly.

Blood pressure GWAS conducted among populations of African origins in the diaspora are rare, and often report different variants associated with BP compared to those reported in non-African populations [13-17]. Attempts to replicate genetic findings in independent populations have returned mixed results [3, 17-19]. The largest African American GWAS of BP included a meta-analysis of 29,378 individuals and identified only one (the SOX6 locus) of the five loci that were associated with BP in a multi-ethnic (African American, European and East Asian) sample of 99,382 individuals [16]. Of the 17 SNPs most strongly associated with BP (p-value $<1x10^{-4}$) in African Americans, three SNPS were replicated (p-value <0.05) in a West African sample [15]. Among Ugandan adults, 11 out of 27 BP related candidate SNPs (selected because of previous association with BP from BP GWASs or admixture mapping analysis) were replicated (p-value <0.05) with eight of the 11 SNPs having the same effect direction as in the discovery sample [19].

Twin studies report that over 30% of BP variability is heritable [20, 21] but established variants associated with BP account for only 2-5% of BP variation [22, 23]. This strongly suggests the existence of important undiscovered variants. This "missing heritability" could be due to rare or to common SNPs, all conferring small increases or decreases in expected BP [24, 25]. There is a need for both GWAS and replication studies to further elucidate and improve generalisability of BP genetic findings.

The role of environmental and of life-style factors for hypertension among Africans is well documented as reviewed in [7, 8]. However, the contribution of genetic variants remains unknown and understudied [12]. It is not clear whether genetic loci associated with hypertension among populations of non-African origin influence susceptibility or

protection to hypertension in populations on the African continent. Independent confirmation is necessary to validate BP SNPs in different populations. We used data from the Entebbe Mother and Baby Study (EMaBS) birth cohort [26] to conduct 1) a GWAS of systolic and diastolic BP and 2) a replication study of candidate SNPs identified in previously published BP GWAS's. The GWAS aimed to identify novel BP loci unique to this population while candidate gene analysis aimed to identify variants influencing BP across different ethnic groups.

We hypothesised that genetic variants (either unique or not unique to populations in Africa) would be associated with BP among Ugandan adolescents. Individuals of African origin have different genetic make-up from other ethnic groups [7-10]. Identifying genetic variants associated with BP enhances our understanding of BP regulation and might highlight potential drug targets for hypertension treatment and prevention. Furthermore, the identification of variants associated with hypertension in both adolescence and adulthood could offer opportunities for early risk prediction.

Methods

The EMaBS [trial registration ISRCTN32849447] in Uganda, was originally designed to investigate the influence of worms and their treatment in pregnancy and early childhood on vaccine response and on infections in childhood [26]. Briefly, between April 2003 and November 2005, 2,507 pregnant women in their second or third trimester were randomised in a 2 x 2 factorial design to receive single-dose albendazole (400 mg) or matching placebo and single-dose praziquantel (40 mg/kg) or matching placebo. At 15 months of age, the resulting 2,345 live-born infants were randomised to receive quarterly albendazole or matching placebo up to 5 years of age.

The offspring continued under follow-up after the trial ended in 2011. From 20th May 2014 to 16th June 2016, cohort participants who were now aged 10-11 years were enrolled in a BP sub-study, as part of which additional anthropometric and BP data were collected [27]. Adolescents were included in this study if they were aged 10 or 11 years and attending their routine annual follow-up during the BP sub-study period (11-year olds who had previously enrolled as 10-year olds were not included twice). Where necessary, enrolment was postponed until the participant was free of malaria (fever or axillary temperature ≥37.5°C and parasitaemia) and other illnesses [27]. BP was measured as previously described [27]. Briefly, on the day of enrolment, after five minutes rest period, trained nurses measured BP thrice five minutes apart, on the right arm supported at the heart level, with the participant seated upright all the way to the back of the chair, with

legs uncrossed and feet flat on the floor. Automated Omron (M6, HEM-700) machines validated every 6 months by the Uganda National Bureau of Standards, were used. Blood pressure phenotypes for this analysis were the mean of the second and third readings for systolic and diastolic BP, i.e. systolic and diastolic BP were analysed as two separate phenotypes. The second and third BP readings were, on average, lower than the first BP reading but similar to each other for both systolic and diastolic BP [27].

Early in 2012, whole-genome genotyping of 1391 EMaBS participants was undertaken. Genotypic data was generated from red cell pellets that had been separated and stored at -80°C until processing. Approximately 2.2 million genetic variants were generated at Wellcome Trust Sanger Institute using the Illumina HumanOmni2.5M-8 ('octo') Beadchip arrays, version 1.1 (Illumina Inc., San Diego, USA). Quality control (using standard pipelines) was performed at the University of Oxford using commands in PLINK (version 1.7) [28], to remove individuals and variants with high levels of missingness or deviations from expected levels of heterozygosity or Hardy-Weinberg equilibrium (p-value $<1x10^{-8}$). Untyped genetic variants and the variants identified for replication analysis were imputed in the EMaBS sample using a merged panel (1000 Genomes [29], African genome variation project [AGVP] [30], and Uganda 2000 Genomes (UG2G), genomes of Ugandan individuals of diverse ethnicity from rural Uganda) at the Wellcome Trust Centre for Human Genomics. SHAPEIT2 (version 2 790) [31] and IMPUTE2 (version 2.3.2) [32] were used for imputation using settings as recommended for African populations. Only SNPs with an INFO score greater than 0.3 and a minor allele frequency greater than 0.01 were taken forward for analysis.

Ethical approvals for the EMaBS, including the genetic study, were granted by the Uganda Virus Research Institute Science and Ethics Committee; the Uganda National Council for Science & Technology; and the London School of Hygiene & Tropical Medicine. The Oxford Tropical Research Ethics Committee provided additional approval for the genetic study. Written informed assent and consent were obtained.

Association analysis

The analysis included all adolescents from EMaBS who had both phenotypic and genotypic data. The two outcomes (mean systolic BP and mean diastolic BP) were analysed separately. GWAS of BP (systolic and diastolic) as quantitative traits was done using mixed linear regression methods (allowing for population substructure) assuming an additive model and controlling for age and body mass index (BMI) as covariates in genome-wide complex trait analysis (GCTA) version 1.22 [33]. A p-value $<5x10^{-8}$ was

considered as the threshold to denote genome-wide significance for SNPs. Results for SNPs with p-value $<1x10^{-6}$ are reported. Manhattan plots and QQ plots were constructed to show the distributions of association p-values and the departure of the observed P-values from the null, respectively.

For the replication component of the study, SNPs from previously published BP GWASs were searched to identify variants reported to be associated with systolic or diastolic BP (p-value $<5x10^{-8}$ in the original GWAS) for the replication study. Replication analysis was conducted using linear regression adjusting for age and BMI in Stata version 14 (College Station, Texas, USA). Variants were tested for association with the phenotype they were associated with in the published GWAS, that is variants associated with systolic BP in a published GWAS were tested for association with systolic BP but not with diastolic BP and vice versa. P-value <0.05 was considered the threshold for statistical significance for the replication study although results were also interpreted in light of a Bonferroni correction allowing for all tests done in the replication analysis. Base pair position is based on the Genome Reference Consortium Human Build 37 (February 2009).

Results

The discovery GWAS analysis used data on 20,074,711 SNPs from 815 adolescents. These adolescents had a mean age of 10.4 years, a mean BMI of 16.0 kg/m^2, mean systolic BP of 106.0 mmHg and mean diastolic BP of 65.3 mmHg. Four hundred and seventeen (51%) of the adolescents were male. Detailed characteristics of EMaBS participants included and not included in the analysis are described in Table S1. Offspring included in the genetic analysis were similar for most characteristics to those not included, except that those included were more likely to have been delivered in Entebbe hospital than elsewhere and to have been exclusively breastfeeding at six weeks of age.

The distributions of association p-values (Manhattan plot) for systolic and diastolic BP phenotypes are shown in Figure 7.2-1and the QQ plots in Figure 7.2-2.

Figures

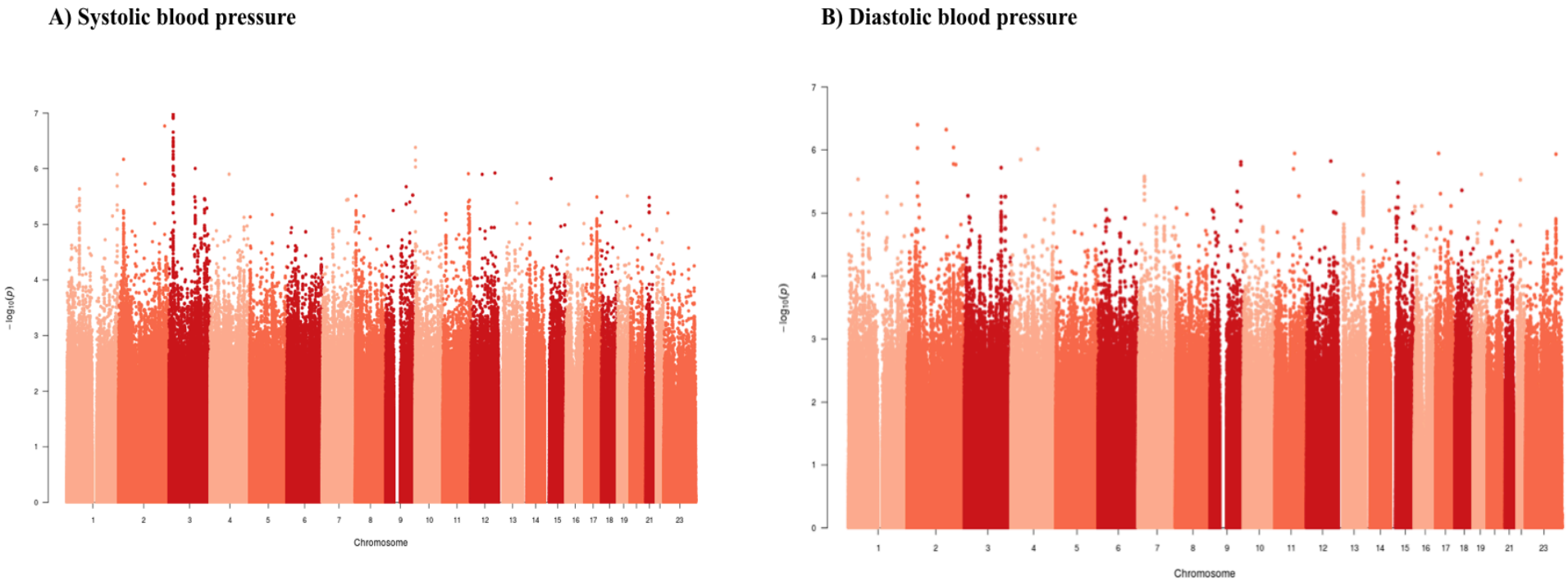

Figure 7.2-1 Manhattan plot for the association of variants with A) systolic blood pressure and B) diastolic blood pressure, adjusting for age and body mass index as fixed covariates

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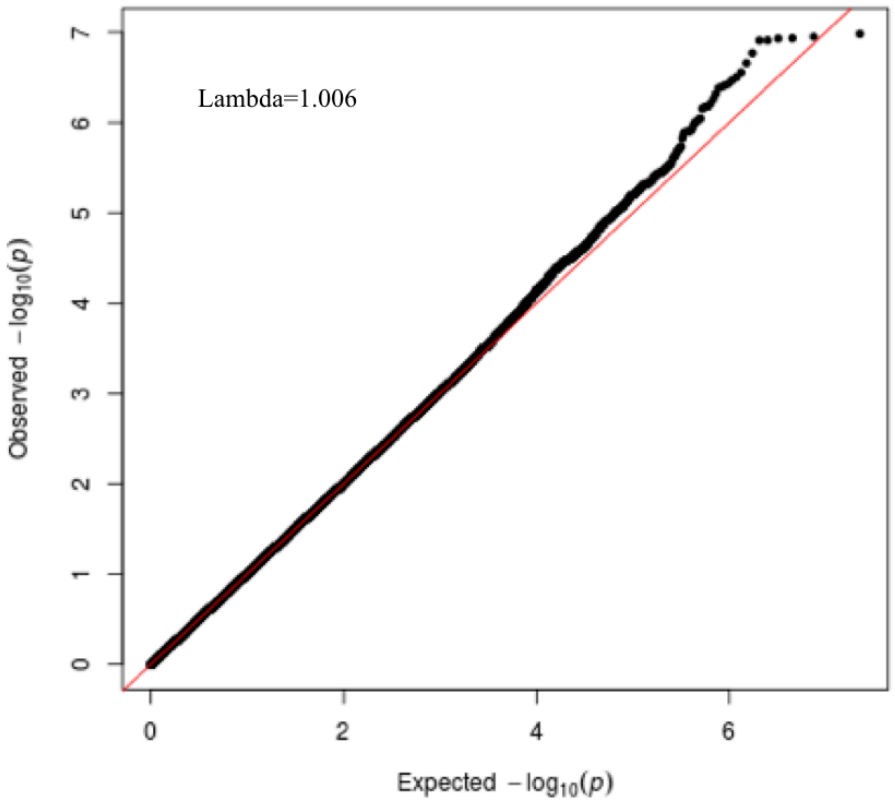
A) Systolic blood pressure



B) Diastolic blood pressure

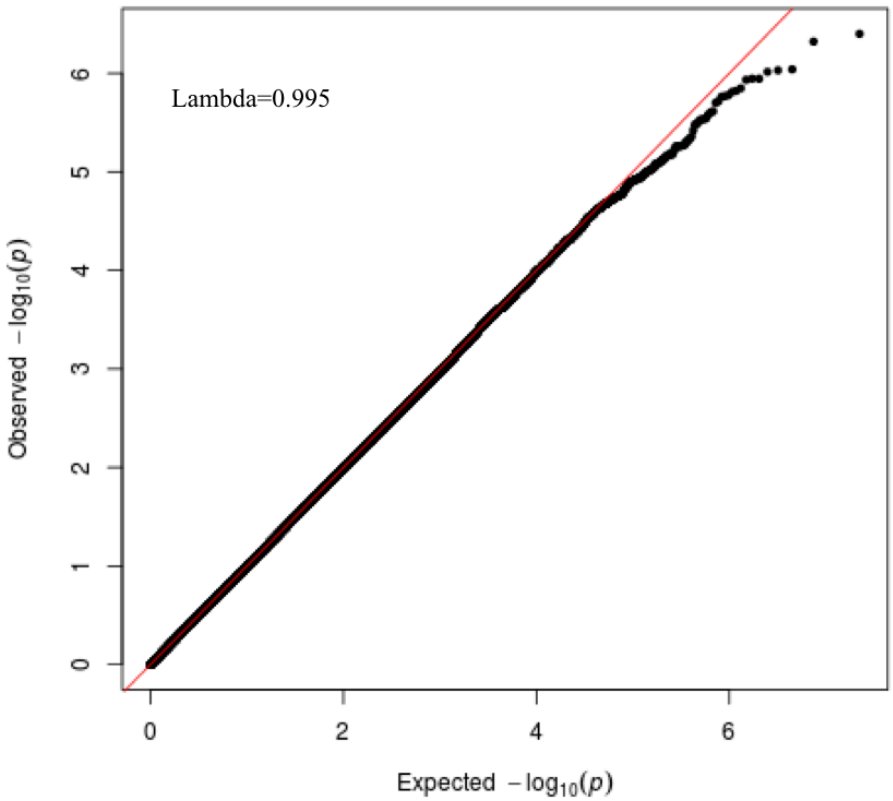


Figure 7.2-2 QQ plot for the two phenotypes and the genomic control coefficient (lambda)

Table 1 Genome-wide association study results: SNPs associated with blood pressure (systolic or diastolic) at p-value < 1.0 x 10^{-7}

Chr	Nearest gene†	Position‡	SNP	Distance to gene (kb)	Type	EA	RA	EAF	β	s.e	P-value
Systolic blood pressure											
2	KLHL29	23904233	rs181430167	0	Intron	C	T	0.05	4.62	9.24	6.8 x 10^{-7}
10	LINC00701	2565732	rs71502208	500	Unknown	A	G	0.22	2.51	5.05	9.3 x 10^{-7}
3	SGOL1/SGOL-ASI	20295100	rs139992073	200	Unknown	C	T	0.14	2.83	5.74	9.7 x 10^{-7}
3	PLXNA1	126737466	rs73861745	0	Intron	A	G	0.26	2.25	4.57	9.9 x 10^{-7}
Diastolic blood pressure											
2	ZFP36L2/THADA/LOC1001297261	43291689	rs12991132	500	Unknown	A	G	0.59	-1.87	3.61	4.0 x 10^{-7}
2	COBLL	165534347	rs111770209	20	Unknown	T	C	0.94	-3.82	7.53	4.8 x 10^{-7}
2	DNAH7	196853830	rs13403027	0	Intron	A	G	0.71	-1.91	3.87	9.1 x 10^{-7}
4	ANK2	114209732	rs29356	0	Intron	T	C	0.69	1.91	3.87	9.6 x 10^{-7}

Chr; chromosomes, SNP; single nucleotide polymorphism, EA; effect allele, RA; reference allele, EAF; effect allele frequency, s.e; standard error, β; effect size estimates correspond to mean difference in mmHg per effect allele for systolic or diastolic blood pressure, adjusted for age and body mass index.

† Named according to the nearest annotated gene(s)

‡ Given with respect to Build 37 (GRCh37/hg19)

Table 2 **Loci associated with systolic blood pressure identified from previous GWAS and results of replication in Entebbe Mother and Baby Study (EMaBS) sample**

						Discovery sample				EMaBS sample			
Chr	**SNP**	**Position**[†]	**Nearest gene**	**Population**	**EA/RA**	**EAF**	**β**	**s.e**	**P-value**	**EAF**	**β**[‡]	**s.e**	**P-value**
1	rs839755	43856410	SZT2	E	A/C	0.62	-0.27	0.03	5.4×10^{-18}	0.81	0.29	0.70	6.8×10^{-1}
1	**rs4926499**	**249155909**	**AL672294.1**	**E**	**C/G**	**0.82**	**0.30**	**0.04**	**1.3×10^{-11}**	**0.98**	**-5.12**	**2.00**	**1.1×10^{-2}**
1	rs1043069	180859368	XPR1	E	T/G	0.62	0.23	0.03	5.2×10^{-14}	0.63	0.02	0.62	9.7×10^{-1}
1	rs4651224	184585182	C1orf21	E	T/C	0.45	0.20	0.03	9.0×10^{-11}	0.94	0.75	1.24	5.5×10^{-1}
1	rs2807337	22577371	WNT4	E	T/C	0.37	0.19	0.03	2.8×10^{-9}	0.43	-0.70	0.58	2.3×10^{-1}
1	**rs7514579**	**94051350**	**BCAR3**	**E**	**A/C**	**0.77**	**0.22**	**0.03**	**5.5×10^{-10}**	**0.50**	**1.25***	**0.67**	**4.0×10^{-2}**
1	rs17396055	94730954	ARHGAP29	E	A/G	0.33	-0.17	0.03	4.0×10^{-8}	0.08	0.80	1.02	4.3×10^{-1}
1	rs12042924	197297417	CRB1	E	T/C	0.53	-0.18	0.03	2.6×10^{-9}	0.86	-1.29	0.86	1.3×10^{-1}
1	rs7555285	209970355	IRF6	E	C/G	0.80	0.23	0.04	1.1×10^{-9}	0.83	-0.52	0.77	5.0×10^{-1}
1	rs33996239	203109801	ADORA1	E	T/C	0.06	-0.37	0.07	3.4×10^{-8}	0.09	-0.13	1.08	9.0×10^{-1}
1	rs2932538	113216543	MOV10	E	G/A	0.75	0.39	-	1.2×10^{-9}	0.85	0.15	0.80	8.6×10^{-1}
1	rs7515635	42408070	HIVEP3	E	T/C	0.47	0.31	0.04	4.8×10^{-12}	0.68	0.08	0.65	9.0×10^{-1}
1	rs17367504	11862778	MTHFR-NPPB	E	G/A	0.14	-0.85	0.11	2.0×10^{-13}	0.09	0.66	1.10	5.5×10^{-1}
1	rs2493292	3328659	PRDM16	E/AA	T/C	0.15	0.37	0.07	1.4×10^{-8}	0.17	-0.36	0.82	6.6×10^{-1}
1	rs880315	10796866	CASZ1	AS	C/T	0.34	1.08	0.03	2.2×10^{-8}	0.11	-0.91	0.96	3.5×10^{-1}
1	rs3820068	15798197	CELA2A	E	A/G	0.81	0.43	0.06	1.1×10^{-8}	0.63	0.81	0.63	2.0×10^{-1}
1	rs10922502	89360158	GTF2B	E	A/G	0.62	-0.38	0.05	2.2×10^{-15}	0.67	-0.32	0.64	6.1×10^{-1}
2	**rs2972146**	**227100698**	**2q36.3**	**E**	**T/G**	**0.65**	**0.17**	**-**	**8.4×10^{-9}**	**0.86**	**-0.19**	**0.86**	**4.0×10^{-2}**
2	rs1446468	164963486	FIGN-GRB14	E	T/C	0.53	-0.50	0.07	1.8×10^{-12}	0.95	0.46	1.34	7.3×10^{-1}
2	rs16849225	164906820	FIGN-GRB14	AS	C/T	0.61	0.75	0.11	3.5×10^{-11}	0.89	-1.04	0.93	2.7×10^{-1}
2	rs7562	28635740	FOSL2	E	T/C	0.52	0.26	0.05	1.9×10^{-8}	0.26	0.20	0.67	7.6×10^{-1}
2	rs13420463	37517566	PRKD3	E	A/G	0.77	0.36	0.05	7.0×10^{-11}	0.45	0.03	0.63	9.6×10^{-1}
2	rs55780018	208526140	METTL21A-AC079767.3	E	T/C	0.54	-0.39	0.05	5.9×10^{-16}	0.75	0.59	0.74	4.2×10^{-1}
2	**rs1275988**	**26914364**	**KCNK3**	**E**	**T/C**	**0.23**	**-0.60**	**0.09**	**2.6×10^{-10}**	**0.14**	**2.01**	**0.85**	**1.8×10^{-2}**
2	**rs6712094**	**165043460**	**FIGN-GRB14**	**E**	**A/G**	**0.70**	**0.60**	**0.10**	**9.9×10^{-9}**	**0.89**	**-3.72**	**1.01**	**2.6×10^{-4}**
2	rs1344653	19730845	OSR1	E/AS	A/G	0.54	-0.27	0.04	7.8×10^{-12}	0.67	0.07	0.66	9.1×10^{-1}
2	rs2300481	66782467	MEIS1	E	T/C	0.39	0.20	0.03	1.6×10^{-10}	0.37	0.73	0.61	2.3×10^{-1}
2	rs35590893	43716933	HADA	E	A/G	0.27	-0.24	0.03	1.7×10^{-12}	0.13	0.52	0.86	5.5×10^{-1}
2	rs67720684	18975439	NT5C1B	E	A/C	0.24	0.19	0.04	3.8×10^{-8}	0.36	-0.41	0.60	5.0×10^{-1}
2	rs28377357	112769721	MERTK	E	A/G	0.29	-0.21	0.03	9.6×10^{-11}	0.31	0.87	0.64	1.8×10^{-1}
2	rs72816333	60096560	RP11-444A22.1	E	A/T	0.83	0.23	0.04	5.5×10^{-9}	0.96	-0.15	1.48	9.2×10^{-1}
2	rs28558491	187816321	ZSWIM2	E	T/C	0.74	-0.21	0.03	7.5×10^{-10}	0.29	0.55	0.67	4.1×10^{-1}
2	rs6723509	122000745	TFCP2L1	E	T/C	0.86	0.25	0.04	7.6×10^{-9}	0.91	0.78	1.00	4.4×10^{-1}
2	rs1044822	230629138	TRIP12	E	T/C	0.15	-0.25	0.04	5.2×10^{-9}	0.09	-0.41	1.07	7.0×10^{-1}

2	rs12694277	213188795	ERBB4	E	T/C	0.30	-0.20	0.03	1.8×10^{-9}	0.62	-0.05	0.61	9.4×10^{-1}
2	rs6739913	185033065	ZNF804A	E	A/G	0.28	0.18	0.03	6.5×10^{-8}	0.21	0.21	0.71	7.6×10^{-1}
2	**rs2920899**	**55279681**	**RTN4**	**E**	**T/G**	**0.79**	**0.20**	**0.04**	**9.5×10^{-8}**	**0.86**	**1.58***	**0.78**	**4.0×10^{-2}**
3	rs9810888	53635595	CACNA1D	AS	G/T	0.39	0.53	0.10	5.5×10^{-8}	0.60	-1.15	0.60	5.4×10^{-2}
3	rs11128722	14958126	FGD5	E	A/G	0.56	-0.31	0.05	3.6×10^{-11}	0.32	0.39	0.10	5.2×10^{-1}
3	rs9859176	134000025	RYK	E	T/C	0.40	0.32	0.05	1.3×10^{-11}	0.17	0.57	0.77	4.6×10^{-1}
3	rs419076	169100886	MECOM	E	T/C	0.47	0.41	-	1.8×10^{-13}	0.57	-0.60	0.61	3.2×10^{-1}
3	rs347591	11290122	HRH1	E/AS/AA	G/T	0.35	-0.53	0.11	1.5×10^{-8}	0.57	-0.41	0.60	4.9×10^{-1}
3	rs13082711	27537909	SL4A7	E	T/C	0.78	-0.24	-	3.8×10^{-9}	0.95	-0.33	1.41	8.1×10^{-1}
3	rs319690	47927484	MAP4	E	T/C	0.50	0.42	0.07	4.7×10^{-8}	0.44	0.72	0.62	2.5×10^{-1}
3	rs12638085	30405936	TGFBR2	E	A/T	0.35	0.22	0.03	5.6×10^{-12}	0.12	-1.31	0.92	1.6×10^{-1}
3	rs6788984	41107173	CTNNB1	E	A/G	0.86	0.30	0.04	3.8×10^{-12}	0.71	-0.05	0.66	9.4×10^{-1}
3	**rs9875380**	**132780356**	**TMEM108**	**E**	**T/C**	**0.46**	**-0.18**	**0.03**	**6.5×10^{-9}**	**0.26**	**-1.55***	**0.68**	**2.3×10^{-2}**
3	rs863930	135949737	PCCB	E	A/C	0.54	0.19	0.03	5.1×10^{-10}	0.63	-0.77	0.62	2.2×10^{-1}
3	rs78151625	158316726	MLF1	E	T/C	0.83	-0.25	0.04	1.6×10^{-9}	-0.82	3.49	3.49	8.1×10^{-1}
3	rs6774721	49381898	ARIH2	E	C/T	0.88	0.28	0.05	6.4×10^{-9}	0.83	-0.94	0.85	3.7×10^{-1}
3	rs9857362	74710462	CNTN3	E	A/C	0.53	0.17	0.03	1.6×10^{-8}	0.83	0.65	0.77	3.9×10^{-1}
4	rs1458038	81164723	FGF5	E	T/C	0.29	0.71	-	1.5×10^{-23}	0.04	1.28	1.53	4.1×10^{-1}
4	rs2291435	38387395	TBC1D1-FLJ13197	E/AA	T/C	0.52	-0.34	0.04	1.9×10^{-14}	0.30	-0.54	0.64	4.0×10^{-1}
4	rs13112725	106911742	NPNT	E	C/G	0.76	0.44	0.06	1.5×10^{-14}	0.61	-0.62	0.60	3.0×10^{-1}
4	rs231708	2694773	FAM193A	E	C/G	0.69	-0.12	0.03	4.7×10^{-18}	0.24	1.14	0.68	9.6×10^{-2}
4	rs7439567	138464842	P11-714L20.1	E	T/C	0.42	0.25	0.03	2.3×10^{-16}	0.81	-0.58	0.76	4.4×10^{-1}
4	rs2610990	18008232	LCORL	E	A/G	0.26	-0.29	0.03	2.8×10^{-17}	0.19	-1.19	0.77	1.2×10^{-1}
4	**rs17035181**	**157678511**	**PDGFC**	**E**	**T/G**	**0.85**	**0.31**	**0.04**	**7.6×10^{-13}**	**0.75**	**-1.47**	**0.71**	**3.8×10^{-2}**
4	rs1347345	95938386	MPR1B	E	A/G	0.62	-0.18	0.03	6.9×10^{-9}	0.87	0.23	0.88	8.0×10^{-1}
4	rs12511987	46595623	GABRA2	E	T/G	0.82	-0.23	0.04	5.4×10^{-9}	0.94	-0.53	1.45	7.2×10^{-1}
4	rs2014912	86715670	ARHGAP24	E/AS	T/C	0.16	0.62	0.08	5.4×10^{-17}	0.16	1.16	0.82	1.6×10^{-1}
5	rs13359291	122476457	PRDM6	E/AS	A/G	0.31	0.53	0.07	8.9×10^{-16}	0.16	0.66	0.77	3.9×10^{-1}
5	rs1173771	32815028	NPR3-C5orf23	E	G/A	0.60	0.50	-	1.8×10^{-16}	0.82	1.09	0.82	1.9×10^{-1}
5	rs11953630	157845402	EBF1	E	T/C	0.37	-0.41	-	3.0×10^{-11}	0.13	-0.34	0.88	7.0×10^{-1}
5	rs10077885	114390121	TRIM36	E	A/C	0.50	-0.28	0.04	1.6×10^{-10}	0.65	0.20	0.63	7.5×10^{-1}
5	rs6595838	127868199	FBN2	E	A/G	0.30	0.34	0.05	7.6×10^{-12}	0.63	0.62	0.59	2.9×10^{-1}
5	rs1173766	32804528	NPR3	AS	C/T	0.60	0.63	0.11	1.9×10^{-8}	0.66	1.13	0.64	7.8×10^{-1}
5	rs10069690	1279790	TERT	E	T/C	0.26	0.31	0.04	4.8×10^{-17}	0.66	-0.54	0.65	4.0×10^{-1}
5	rs709668	96174186	CTD-2260A17.2	E	A/G	0.20	-0.29	0.04	6.0×10^{-15}	0.40	-0.43	0.59	4.7×10^{-1}
5	rs246973	68007803	SLC30A5	E	T/C	0.29	0.25	0.03	1.5×10^{-13}	0.40	2.51	1.53	1.0×10^{-1}
5	rs702395	140086677	ZMAT2	E	T/C	0.44	0.23	0.03	3.5×10^{-14}	0.29	-0.15	0.65	8.2×10^{-1}

5	rs13179413	55868097	AC022431.2	E	T/C	0.28	0.22	0.03	1.1×10^{-10}	0.21	-0.23	0.72	7.5×10^{-1}
5	**rs62373688**	**127352807**	**CTC-228N24.3**	**E**	**A/T**	**0.13**	**0.27**	**0.04**	**1.5×10^{-9}**	**0.24**	**1.40***	**0.70**	**4.6×10^{-2}**
5	rs74774746	33411769	TARS	E	C/G	0.26	-0.19	0.04	5.6×10^{-8}	0.14	1.49	0.88	9.2×10^{-2}
5	rs1008058	122435627	PRDM6	E	A/G	0.14	0.55	-	3.0×10^{-10}	0.13	0.31	0.89	7.3×10^{-1}
6	rs79030490	134087689	TARID-TCF21	AA	A/C	0.09	-1.83	0.31	3.0×10^{-9}	0.11	0.99	1.19	4.0×10^{-1}
6	rs76987554	134080855	TARID-TCF21	AA	C/T	0.91	1.85	0.31	2.2×10^{-9}	0.90	-0.88	1.20	4.6×10^{-1}
6	rs1799945	26091179	HFE	E	G/C	0.14	0.63	-	7.7×10^{-12}	0.02	-1.84	2.17	4.0×10^{-1}
6	**rs805303**	**31616366**	**BAT2-BAT5**	**E**	**G/A**	**0.61**	**0.38**	**-**	**1.5×10^{-11}**	**0.40**	**1.66***	**0.61**	**7.0×10^{-3}**
6	rs6911827	22130601	CASC15	E	T/C	0.45	0.30	0.05	2.0×10^{-10}	0.82	0.81	0.81	3.2×10^{-1}
6	rs2270860	43270151	SLC22A7	E/AA	T/C	0.37	0.32	0.05	2.9×10^{-11}	0.78	-1.20	0.71	9.1×10^{-2}
6	**rs1563788**	**43308363**	**TTBK1-SLC22A7-ZNF318**	**E/AS**	**T/C**	**0.31**	**0.51**	**0.06**	**2.2×10^{-16}**	**0.78**	**-1.46**	**0.71**	**4.0×10^{-2}**
6	rs13209747	127115454	RSPO3	E/AA/AS	T/C	0.19	0.85	0.21	2.6×10^{-10}	0.07	-0.13	1.09	9.1×10^{-1}
6	rs17080102	151004770	PLEKHG1	E/AA/AS	C/G	0.10	-1.02	0.25	4.8×10^{-8}	0.15	-0.61	0.81	4.5×10^{-1}
6	rs9368222	20686996	CDKAL1	E	A/C	0.27	0.23	0.03	1.8×10^{-11}	0.17	-0.07	0.81	9.3×10^{-1}
6	rs10782230	126228512	NCOA7	E	A/G	0.48	0.21	0.03	2.9×10^{-12}	0.41	0.71	0.62	2.6×10^{-1}
6	rs2745599	1613686	FOXC1	E	A/G	0.55	0.22	0.03	9.8×10^{-12}	0.10	-1.48	0.95	1.2×10^{-1}
6	rs9885632	131311909	EPB41L2	E	T/C	0.73	0.24	0.03	4.3×10^{-12}	0.94	0.64	1.39	6.4×10^{-1}
6	rs7763294	140383733	CITED2	E	T/G	0.32	-0.20	0.03	6.4×10^{-10}	0.10	0.32	0.97	7.4×10^{-1}
7	rs2969070	2512545	CHST12-LFNG	E	A/G	0.63	-0.30	0.05	1.4×10^{-10}	0.97	-0.01	1.80	1.0×10^{-0}
7	rs11556924	129663496	ZC3HC1	E	T/C	0.38	-0.28	0.05	7.6×10^{-9}	0.01	-0.24	1.58	8.8×10^{-1}
7	rs13238550	131059056	MKLN1	E	A/G	0.40	0.33	0.05	1.9×10^{-12}	0.09	-0.14	1.14	9.0×10^{-1}
7	rs1011018	139463264	HIPK2	E	A/G	0.20	-0.33	0.06	1.5×10^{-8}	0.61	-0.17	0.59	7.8×10^{-1}
7	rs4728142	128573967	7q32.1	E	A/G	0.43	-0.24	-	3.5×10^{-8}	0.23	-0.27	0.69	7.0×10^{-1}
7	rs17477177	106411858	PIK3CG	E	T/C	0.72	-0.55	0.08	5.7×10^{-11}	0.94	-0.86	1.15	4.5×10^{-1}
7	rs17428471	27337867	EVX1-HOXA	E/AA/AS	T/G	0.14	1.20	0.24	2.1×10^{-12}	0.13	1.39	0.90	1.3×10^{-1}
7	rs11563582	27351650	EVX1-HOXA	AA	A/G	0.13	1.61	0.28	7.1×10^{-9}	0.17	0.56	0.81	4.9×10^{-1}
7	rs848445	77572461	PHTF2	E	T/C	0.23	-0.20	0.03	2.3×10^{-9}	0.08	-0.09	1.03	9.3×10^{-1}
7	rs6963105	75097488	POM121C	E	A/G	0.43	-0.19	0.03	3.8×10^{-9}	0.06	0.17	1.15	8.8×10^{-1}
7	rs10274928	28142088	JAZF1	E	A/G	0.49	0.16	0.03	8.2×10^{-8}	0.66	-0.05	0.63	9.3×10^{-1}
7	rs11771693	150050111	RARRES2	E	A/G	0.67	0.18	0.03	1.9×10^{-8}	0.52	-0.25	0.62	6.8×10^{-1}
8	rs4841569	11452177	BLK-GATA4	E/AS	G/A	0.51	0.47	0.02	5.6×10^{-10}	0.91	0.33	1.13	7.7×10^{-1}
8	rs2898290	11433909	BLK-GATA4	E	T/C	0.53	0.53	0.80	3.2×10^{-8}	0.61	-0.71	0.60	2.3×10^{-1}
8	**rs1986971**	**10268736**	**MSRA**	**E**	**A/G**	**0.70**	**0.26**	**0.03**	**1.6×10^{-14}**	**0.80**	**1.45***	**0.73**	**4.8×10^{-2}**
8	rs1906672	38130025	WHSC1L1	E	A/G	0.23	0.30	0.04	1.2×10^{-16}	0.16	0.08	0.82	9.2×10^{-1}
8	rs72688070	81393697	Y_RNA	E	T/C	0.17	-0.27	0.04	2.8×10^{-11}	0.44	-0.62	0.60	3.0×10^{-1}
8	**rs62491354**	**9730663**	**TNKS**	**E**	**A/G**	**0.13**	**0.31**	**0.04**	**3.3×10^{-12}**	**0.13**	**-2.38**	**0.80**	**3.0×10^{-3}**
8	rs4129585	143312933	TSNARE1	E	A/C	0.44	0.19	0.03	1.0×10^{-9}	0.06	-1.10	1.18	3.5×10^{-1}

8	rs6557876	25900675	EBF2	E	C/T	0.25	-0.37	0.05	2.8×10^{-14}	0.50	-0.20	0.60	7.4×10^{-1}
8	rs894344	135612745	ZFAT	E	A/G	0.60	-0.26	0.05	3.2×10^{-8}	0.65	-0.17	0.64	8.0×10^{-1}
9	rs10760117	123586737	PSMD5	E	T/G	0.42	0.28	0.05	6.1×10^{-10}	0.78	-1.39	0.72	5.3×10^{-2}
9	rs1332813	9350706	PTPRD	E	T/C	0.35	0.22	0.03	2.3×10^{-12}	0.36	0.02	0.62	9.7×10^{-1}
9	**rs7045409**	**95201540**	**CENPP**	**E**	**A/T**	**0.37**	**-0.19**	**0.03**	**2.6×10^{-9}**	**0.90**	**2.16**	**0.94**	**2.2×10^{-2}**
9	rs1891730	130309028	FAM129B	E	T/C	0.62	-0.18	0.03	7.7×10^{-9}	0.39	-1.13	0.61	6.2×10^{-2}
9	rs28558845	4334791	GLIS3	E	C/G	0.16	-0.26	0.04	1.2×10^{-9}	0.24	0.29	0.72	6.9×10^{-1}
10	rs1133400	134459388	INPP5A	E	A/G	0.79	-0.30	0.04	2.5×10^{-15}	0.86	0.11	0.88	9.0×10^{-1}
10	rs11191548	104846178	CYP17A1-NT5C2	E	T/C	0.91	1.16	0.12	7.0×10^{-24}	0.98	-0.41	1.62	8.0×10^{-1}
10	**rs112184198**	**102604514**	**PAX2**	**E**	**A/G**	**0.10**	**-0.66**	**0.08**	**3.6×10^{-18}**	**0.06**	**3.67**	**1.37**	**8.0×10^{-3}**
10	rs1813353	18707448	CACNB2	E	T/C	0.68	0.57	-	2.6×10^{-12}	0.84	-0.74	0.84	3.8×10^{-1}
10	rs932764	95895940	PLCE1	E	G/A	0.44	0.48	-	7.1×10^{-16}	0.15	0.59	0.85	4.9×10^{-1}
10	rs1801253	115805056	ADRB1	E	G/C	0.27	-0.57	0.09	4.7×10^{-10}	0.34	0.21	0.64	7.5×10^{-1}
10	rs4387287	105677897	OBFC1	E/AS	A/C	0.16	0.36	-	9.1×10^{-10}	0.73	-0.00	0.67	1.0×10^{-0}
10	rs4590817	63467553	C10orf107	E	G/C	0.84	0.65	-	4.0×10^{-12}	0.84	-0.55	0.78	4.8×10^{-1}
10	rs7912283	133773019	PPP2R2D	E	A/G	0.35	0.21	0.03	6.4×10^{-11}	0.89	-0.31	0.89	7.3×10^{-1}
10	rs12572586	74751579	PLA2G12B	E	T/C	0.94	-0.39	0.06	1.2×10^{-9}	0.95	1.91	1.41	1.8×10^{-1}
10	rs11197813	118523933	HSPA12A	E	A/G	0.70	-0.18	0.03	3.5×10^{-8}	0.82	0.97	0.74	1.9×10^{-1}
10	rs4373814	18419972	CACNB2	E	G/C	0.55	-0.37		4.8×10^{-11}	0.39	-0.82	0.60	1.7×10^{-1}
11	rs7103648	47461783	RAPSN-PSMC3-SLC39A13	E	A/G	0.61	-0.33	0.05	4.4×10^{-13}	0.85	0.97	0.85	2.6×10^{-1}
11	rs751984	61278246	LRRC10B	E	T/C	0.88	0.41	0.07	3.8×10^{-9}	0.79	0.04	0.72	9.5×10^{-1}
11	rs661348	1905292	LSP1-TNNT3	E	T/C	0.57	-0.65	0.11	7.0×10^{-10}	0.86	0.89	0.98	3.3×10^{-1}
11	rs7129220	10350538	ADM	E	G/A	0.89	-0.62	-	3.0×10^{-12}	0.92	-1.02	1.16	3.8×10^{-1}
11	rs633185	100593538	FLJ32810-TMEM133	E	G/C	0.28	-0.57	-	1.2×10^{-17}	0.23	-0.92	0.71	2.0×10^{-1}
11	rs4757391	16302939	SOX6	E/AS/AA	T/C	0.21	0.56	0.12	5.7×10^{-10}	0.74	0.19	0.65	7.7×10^{-1}
11	rs11229457	58207203	OR5B12	E/AS	T/C	0.24	-0.31	-	2.7×10^{-8}	0.29	-0.36	0.62	5.6×10^{-1}
11	rs381815	16902268	PLEKHA7	E	T/C	0.26	0.57	-	5.3×10^{-11}	0.26	-0.27	0.71	5.6×10^{-1}
11	rs3741378	65408937	RELA	E	T/C	0.14	-0.55	-	3.4×10^{-10}	0.76	-1.04	0.69	1.3×10^{-1}
11	rs4385883	51539339	TRIM48	E	T/A	0.29	-0.25	0.04	1.4×10^{-12}	0.53	-0.10	0.60	8.6×10^{-1}
11	rs11041530	7701503	CYB5R2	AA	C/G	0.11	-1.35	0.25	4.0×10^{-8}	0.17	0.49	0.82	5.5×10^{-1}
11	rs1401454	16250183	SOX6	AA	T/C	0.46	0.55	0.16	5.6×10^{-8}	0.46	-0.60	0.59	3.1×10^{-1}
11	rs7941684	5532222	UBQLN3	AA	T/G	0.80	-1.23	0.22	2.4×10^{-8}	0.82	-0.69	0.80	3.8×10^{-1}
11	rs11031051	30355707	ARL14EP	E	A/C	0.69	-0.22	0.03	7.7×10^{-12}	0.58	0.26	0.61	6.7×10^{-1}
11	**rs67976715**	**68023742**	**C11orf24**	**E**	**C/G**	**0.23**	**0.21**	**0.04**	**6.8×10^{-9}**	**0.07**	**-2.44**	**1.23**	**4.8×10^{-2}**
11	rs10743086	8774923	ST5	E	A/G	0.21	-0.21	0.04	3.6×10^{-8}	0.33	0.96	0.61	1.2×10^{-1}
12	rs11067763	116198341	MED13L	AS	A/G	0.62	0.81	0.10	5.7×10^{-16}	0.78	0.42	0.68	5.4×10^{-1}
12	rs10858966	90567026	ATP2B1	E	C/G	0.29	0.26	0.03	9.2×10^{-15}	0.02	0.86	2.17	6.9×10^{-1}

12	rs2024385	12888438	APOLD1	E	A/T	0.42	-0.26	0.03	5.9×10^{-18}	0.46	-0.57	0.59	3.4×10^{-1}
12	rs11571376	1059556	RAD52	E	C/G	0.70	-0.18	0.03	5.7×10^{-8}	0.73	-0.49	0.71	4.9×10^{-1}
12	rs6487543	26438189	SSPN	E	A/G	0.77	0.30	0.05	6.3×10^{-10}	0.08	-0.41	1.05	7.0×10^{-1}
12	**rs2681492**	**90013089**	**ATP2B1**	**E/AS/AA**	**G/A**	**0.17**	**-0.97**	**0.16**	**5.8×10^{-8}**	**0.15**	**1.84**	**0.79**	**2.1×10^{-2}**
12	rs10850411	115387796	TBX5-TBX3	E	T/C	0.70	0.35	-	5.4×10^{-8}	0.61	-0.45	0.60	4.5×10^{-1}
12	**rs17249754**	**90060586**	**ATP2B1**	**E**	**G/A**	**0.84**	**0.93**	**-**	**1.8×10^{-18}**	**0.85**	**-1.58**	**0.80**	**4.8×10^{-2}**
12	rs10437954	58003922	ARHGEF25	E	A/G	0.90	-0.41	0.05	1.6×10^{-14}	0.67	0.61	0.66	3.5×10^{-1}
12	rs5742643	102837863	IGF1	E	A/G	0.25	-0.22	0.03	2.0×10^{-10}	0.26	-0.67	0.69	3.3×10^{-1}
12	rs7963801	79685226	SYT1	E	T/C	0.41	-0.24	0.03	2.8×10^{-14}	0.01	3.33	2.54	1.9×10^{-1}
12	rs7976167	24210599	SOX5	E	T/C	0.69	0.18	0.03	3.8×10^{-8}	0.84	1.61	0.82	5.1×10^{-2}
13	rs9532243	32191408	RXFP2	E	A/C	0.48	0.22	0.03	8.2×10^{-14}	0.55	-0.33	0.59	5.8×10^{-1}
13	rs606950	22298923	FGF9	E	A/G	0.62	0.27	0.03	3.2×10^{-18}	0.47	0.88	0.60	1.4×10^{-1}
13	rs78474310	73826901	KLF5	E	A/G	0.96	-0.47	0.07	1.5×10^{-10}	0.99	-0.72	3.38	8.3×10^{-1}
13	rs9526707	51489186	RNASEH2B	E	A/G	0.32	-0.20	0.03	2.7×10^{-10}	0.12	-1.59	0.91	8.0×10^{-2}
13	rs9549328	113636156	MCF2L	E	T/C	0.23	0.32	0.06	1.5×10^{-8}	0.15	0.59	0.89	5.1×10^{-1}
14	rs8014182	103859962	MARK3	E	T/C	0.14	-0.33	0.04	5.2×10^{-14}	0.02	0.19	1.93	9.2×10^{-1}
14	rs11159091	75074316	LTBP2	E	A/G	0.46	0.20	0.03	6.7×10^{-11}	0.01	2.00	2.77	4.7×10^{-1}
14	rs11623535	72462381	RGS6	E	A/G	0.74	0.21	0.03	1.0×10^{-9}	0.57	0.71	0.60	2.4×10^{-1}
14	rs17115145	30122409	PRKD1	E	T/C	0.40	0.18	0.03	7.4×10^{-9}	0.57	-0.33	0.58	5.7×10^{-1}
14	rs9888615	53377540	FERMT2	E	T/C	0.29	-0.32	0.05	3.5×10^{-10}	0.62	0.38	0.63	5.4×10^{-1}
14	rs8016306	63928546	PPP2R5E	E	A/G	0.80	0.34	0.06	3.7×10^{-9}	0.15	-0.10	0.84	9.1×10^{-1}
15	rs1563894	68635775	TGA11	E	A/G	0.19	-0.09	-	2.9×10^{-8}	0.69	0.17	0.63	7.8×10^{-1}
15	rs2521501	91437388	FURIN-FES	E	T/A	0.31	0.65	-	5.2×10^{-9}	0.21	-0.39	0.76	6.1×10^{-1}
15	rs1378942	75077367	CYP1A1-ULK3	E	C/A	0.35	0.61	-	5.7×10^{-23}	0.97	-0.03	1.70	9.9×10^{-1}
15	rs35199222	81013037	ABHD17C	E	A/G	0.45	0.32	0.05	5.2×10^{-12}	0.07	0.39	1.18	7.5×10^{-1}
15	rs11632436	86295286	RP11-158M2.4	E	C/G	0.50	0.22	0.03	2.0×10^{-13}	0.21	-0.31	0.73	6.7×10^{-1}
15	rs3743157	85680532	PDE8A	E	A/C	0.17	0.29	0.04	4.2×10^{-13}	0.71	0.10	0.66	8.8×10^{-1}
15	rs4965529	100145224	MEF2A	E	T/G	0.17	-0.26	0.04	5.4×10^{-11}	0.31	-0.16	0.64	8.0×10^{-1}
16	rs11639856	24788645	TNRC6A	E/AA	A/T	0.19	-0.34	0.06	1.3×10^{-8}	0.19	0.90	0.78	2.5×10^{-1}
16	rs11643209	75331044	CFDP1	E	T/G	0.42	-0.34	0.05	1.8×10^{-12}	0.74	0.08	0.70	9.1×10^{-1}
16	rs7187540	85318302	LINC00311	E	A/C	0.34	-0.20	0.03	1.0×10^{-8}	0.15	0.01	0.89	9.9×10^{-1}
17	rs4925159	18185510	TOP3A	E	A/G	0.43	0.22	0.03	9.7×10^{-13}	0.73	0.29	0.63	6.5×10^{-1}
17	rs34430710	56876627	PPM1E	E	A/T	0.68	-0.21	0.03	5.0×10^{-11}	0.89	0.95	0.89	2.9×10^{-1}
17	rs1036902	58950791	BCAS3	E	T/C	0.84	-0.25	0.04	1.7×10^{-9}	0.19	-0.14	0.73	8.5×10^{-1}
17	rs1551355	30032420	RP11-805L22.1	E	T/C	0.23	0.21	0.04	3.9×10^{-9}	0.03	-0.05	1.94	9.8×10^{-1}
17	rs12940887	47402807	ZNF652	E	T/C	0.38	0.36	-	1.8×10^{-10}	0.07	-0.48	1.43	7.4×10^{-1}
17	rs57927100	75317300	SEPT9	E	C/G	0.26	-0.31	0.05	4.0×10^{-14}	0.78	-0.60	0.70	3.9×10^{-1}

17	rs2467099	73949045	ACOX1	E	T/C	0.22	-0.30	0.06	3.3×10^{-8}	0.12	-0.82	0.95	3.9×10^{-1}
17	rs12941318	1333598	CRK	E	T/C	0.49	-0.27	0.05	2.5×10^{-8}	0.75	0.67	0.66	3.1×10^{-1}
17	rs12946454	43208121	PLCD3	E	T/A	0.28	0.50	0.17	1.0×10^{-8}	0.06	-0.06	1.20	9.6×10^{-1}
17	rs7406910	46688256	HOXB7	E/AS	T/C	0.12	-0.46	-	3.8×10^{-8}	0.26	0.38	0.65	5.6×10^{-1}
17	rs112280096	79367409	RP11-1055B8.6	E	A/C	0.36	-0.20	0.04	1.3×10^{-9}	0.06	0.68	1.45	6.4×10^{-1}
18	rs12958173	42141977	SETBP1	E	A/C	0.31	0.36	0.05	1.4×10^{-13}	0.32	0.72	0.63	2.6×10^{-1}
18	rs12454712	60845884	BCL2	E	T/C	0.62	0.19	0.03	5.8×10^{-9}	0.76	0.86	0.70	2.2×10^{-1}
18	rs10048404	54578482	WDR7	E	T/C	0.37	-0.26	0.03	1.9×10^{-16}	0.05	-1.12	1.33	4.9×10^{-1}
18	rs11876341	48799991	MEX3C	E	A/G	0.69	-0.21	0.03	1.8×10^{-10}	0.94	2.01	1.48	1.8×10^{-1}
19	rs4247374	7252756	INSR	E	T/C	0.14	-0.59	0.08	1.2×10^{-18}	0.02	-0.93	2.23	6.7×10^{-1}
20	rs1327235	10969030	JAG1	E	G/A	0.46	0.34	-	1.9×10^{-8}	0.59	-0.03	0.59	9.6×10^{-1}
20	rs6015450	57751117	GNAS-EDN3	E	G/A	0.12	0.90	-	3.9×10^{-23}	0.18	0.51	0.74	5.0×10^{-1}
21	rs12627651	44760603	CRYAA-SIK1	E	A/G	0.29	0.39	0.05	2.6×10^{-14}	0.07	1.70	1.28	5.9×10^{-1}
22	rs4823006	29451671	ZNRF3	E/AA	G/A	0.42	-0.26	0.05	7.9×10^{-9}	0.40	0.86	0.58	1.4×10^{-1}
22	rs28578714	50727921	PLXNB2	E	T/C	0.61	0.21	0.03	2.5×10^{-10}	0.53	-0.59	0.58	3.1×10^{-1}

Chr; chromosomes, SNP; single nucleotide polymorphism, E; European ancestry, AA; African ancestry, AS; Asian ancestry, EA; effect allele, RA; reference allele, EAF; effect allele frequency, β; Effect size estimates correspond to mean difference in mmHg per effect allele for systolic or diastolic blood pressure, adjusted for age and body mass index, s.e; standard error

[†] Given with respect to Build 37 (GRCh37/hg19)

[‡] Adjusted for age and body mass index

Bold indicate p-value $<5.0 \times 10^{-2}$ for replication analysis

* Indicate same β direction in both the discovery and EMaBS populations

Table 3 Loci associated with diastolic blood pressure identified from previous GWAS and results of replication in Entebbe Mother and Baby Study (EMaBS) sample

						Discovery sample				EMaBS sample			
Chr	**SNP**	**Position†**	**Nearest gene**	**Population**	**EA/RA**	**EAF**	**β**	**s.e**	**P-value**	**EAF**	**β‡**	**s.e**	**P-value**
1	rs17367504	11862778	MTHFR/NPPB	E	G/A	0.15	-0.55	-	3.5×10^{-19}	0.09	-0.53	0.97	5.8×10^{-1}
1	**rs2169137**	**204497913**	**MDM4**	**E/AS/AA**	**G/C**	**0.27**	**-0.36**	**0.07**	**5.9×10^{-8}**	**0.23**	**1.32**	**0.61**	**3.1×10^{-2}**
1	rs13306561	11865804	MTHFR	E/AS/AA	G/A	0.15	-0.52	0.09	3.0×10^{-19}	0.27	0.04	0.58	9.4×10^{-1}
1	rs2932538	113216543	MOV10	E	G/A	0.75	0.24	-	9.9×10^{-10}	0.85	-0.25	0.70	3.6×10^{-1}
1	rs4846049	11850365	MTHFR-NPPB	E	T/G	0.33	-0.55	0.10	6.7×10^{-8}	0.51	0.72	0.50	1.6×10^{-1}
1	rs6686889	25030470	chr1mb25	E	T/C	0.25	0.19	0.03	3.6×10^{-9}	0.36	-0.28	0.53	5.9×10^{-1}
1	s12405515	172357441	DNM3	E	T/G	0.56	-0.17	0.03	1.4×10^{-9}	0.20	0.68	0.66	3.0×10^{-1}
1	s12408022	217718789	GPATCH2	E	T/C	0.26	0.20	0.03	2.4×10^{-10}	0.06	-0.43	1.14	7.1×10^{-1}
1	rs10916082	227252626	CDC42BPA	E	A/G	0.73	-0.18	0.03	8.4×10^{-9}	0.52	-0.06	0.52	9.1×10^{-1}
1	rs2760061	228191075	WNT3A	E	A/T	0.47	0.23	0.03	2.1×10^{-16}	0.62	-0.02	0.56	9.7×10^{-1}
1	rs953492	243471192	SDCCAG8	E	A/G	0.46	0.22	0.03	7.4×10^{-16}	0.68	-0.18	0.56	7.5×10^{-1}
1	rs2004776	230848702	AGT	E	T/C	0.23	0.32	0.06	5.0×10^{-8}	0.52	-0.45	0.52	3.9×10^{-1}
1	rs1565716	29549216	MECR	E	A/G	0.07	0.21	0.03	3.5×10^{-10}	0.09	0.66	1.02	5.2×10^{-1}
1	rs35981664	218549354	TGFB2	E	A/T	0.69	-0.16	0.02	2.0×10^{-17}	0.99	-0.58	3.69	8.8×10^{-1}
1	rs12142296	46541679	PIK3R3	E	T/G	0.86	-0.16	0.03	8.9×10^{-11}	0.98	1.09	1.66	5.1×10^{-1}
1	rs72704264	145713305	CD160	E	C/G	0.21	0.12	0.02	3.6×10^{-8}	0.03	0.58	1.44	6.9×10^{-1}
2	rs1446468	164963486	FIGN-GRB14	E	T/C	0.53	-0.50	0.07	6.9×10^{-9}	0.95	-0.61	1.17	6.1×10^{-1}
2	rs16823124	183224127	PDE1A	E	A/G	0.42	0.26	0.04	2.0×10^{-10}	0.11	-0.06	0.80	9.4×10^{-1}
2	**rs55701159**	**25139596**	**ADCY3**	**E**	**T/G**	**0.89**	**0.29**	**0.04**	**7.2×10^{-11}**	**0.89**	**-1.98**	**0.79**	**1.3×10^{-2}**
2	rs4952611	40567743	SLC8A1	E	T/C	0.58	-0.16	0.03	4.0×10^{-8}	0.72	-0.08	0.59	9.0×10^{-1}
2	rs2579519	96675166	GPAT2-FAHD2CP	E	T/C	0.63	-0.20	0.03	4.8×10^{-12}	0.72	0.01	0.61	9.9×10^{-1}
2	rs7592578	191439591	TMEM194B	E	T/G	0.19	-0.24	0.04	9.5×10^{-12}	0.27	-0.81	0.61	1.8×10^{-1}
2	rs1063281	218668732	TNS1	E	T/C	0.60	-0.20	0.03	1.3×10^{-12}	0.59	0.28	0.55	6.1×10^{-1}
2	rs1975487	55809054	PNPT1	E	A/G	0.46	-0.16	0.03	1.8×10^{-9}	0.68	0.02	0.55	9.7×10^{-1}
2	rs1220128	158499902	ACVR1C	E	C/G	0.85	0.19	0.02	6.2×10^{-15}	0.40	0.20	0.53	7.1×10^{-1}
2	rs1996992	219651349	CYP27A1	E	T/G	0.05	-0.30	004	4.7×10^{-14}	0.09	-1.00	0.93	2.8×10^{-1}
2	rs13001283	127183454	GYPC	E	A/G	0.16	0.15	0.02	1.9×10^{-10}	0.11	0.81	0.79	3.1×10^{-1}
2	rs7606205	144146311	ARHGAP15	E	A/C	0.70	-0.13	0.02	2.4×10^{-11}	0.49	-0.40	0.53	4.5×10^{-1}
2	rs34570306	146272860	ZEB2	E	T/C	0.53	-0.12	0.02	1.2×10^{-11}	0.07	-0.00	1.04	1.0×10^{-1}
2	rs4851462	98357163	ZAP70	E	T/C	0.63	-0.12	0.02	4.0×10^{-11}	0.90	-0.46	0.91	6.2×10^{-1}
2	rs2707238	38094149	LINC00211	E	C/G	0.28	0.10	0.02	6.8×10^{-8}	0.28	-0.42	0.60	4.8×10^{-1}
3	rs11128722	14958126	FGD5	E	A/G	0.56	-0.17	0.03	5.1×10^{-10}	0.32	0.55	0.53	3.0×10^{-1}
3	rs918466	64710253	ADAMTS9	E	A/G	0.41	-0.18	0.03	1.7×10^{-11}	0.90	0.57	0.86	5.1×10^{-1}
3	rs36022378	49913705	CAMKV-ACTBP13	E	T/C	0.80	-0.20	0.03	4.7×10^{-9}	0.98	-0.37	2.44	8.8×10^{-1}

3	rs743757	50476378	CACNA2D2	E	C/G	0.14	0.25	0.04	2.4×10^{-10}	0.55	-0.60	0.54	2.7×10^{-1}
3	s9827472	56726646	FAM208A	E	T/C	0.37	-0.18	0.03	4.3×10^{-10}	0.48	0.19	0.52	7.0×10^{-1}
3	rs2306374	138119952	MRAS	E	T/C	0.84	-0.18	0.03	7.4×10^{-9}	0.96	2.03	1.37	1.4×10^{-1}
3	rs12374077	185317674	SENP2	E	C/G	0.35	0.16	0.03	9.2×10^{-9}	0.47	0.52	0.52	3.2×10^{-1}
3	rs143112823	154707967	RP11-439C8.2	E	A/G	0.09	-0.40	0.05	1.4×10^{-14}	0.09	-0.41	0.99	6.8×10^{-1}
3	rs419076	169100886	MECOM	E	T/C	0.47	0.24	-	2.1×10^{-12}	0.57	-0.29	0.54	5.8×10^{-1}
3	rs319690	47927484	MAP4	E	T/C	0.50	0.28	0.05	1.8×10^{-8}	0.44	0.26	0.55	6.3×10^{-1}
3	rs1706003	194299967	TMEM44	E	T/G	0.47	0.12	0.02	5.8×10^{-12}	0.01	-3.51	2.17	1.1×10^{-1}
3	rs11923667	101268080	TRMT10C	E	A/T	0.41	0.12	0.02	3.1×10^{-11}	0.27	-1.06	0.57	6.5×10^{-2}
3	rs6777317	197070959	DLG1	E	A/G	0.92	0.12	0.02	1.5×10^{-10}	0.90	0.76	0.92	4.1×10^{-1}
3	rs4634143	23163749	UBE2E2	E	T/C	0.30	0.12	0.02	7.9×10^{-10}	0.07	0.10	1.06	9.3×10^{-1}
3	rs3774372	41877414	ULK4	E	T/C	0.83	-0.37	-	9.0×10^{-14}	0.77	-0.72	0.59	2.2×10^{-1}
4	rs13139571	156645513	GUCY1A3-GUCY1B3	E	C/A	0.76	0.26	-	2.2×10^{-10}	0.86	0.89	0.75	2.4×10^{-1}
4	rs6825911	111381638	ENPEP	AS	C/T	0.51	0.39	0.07	9.0×10^{-9}	0.61	0.46	0.55	4.0×10^{-1}
4	rs2291435	38387395	TBC1D1-FLJ13197	E/AA	T/C	0.52	-0.16	0.03	4.3×10^{-9}	0.30	0.07	5.58	9.0×10^{-1}
4	rs66887589	120509279	PDE5A	E	T/C	0.52	-0.22	0.03	3.4×10^{-15}	0.62	-0.46	0.55	4.0×10^{-1}
4	rs1458038	81164723	FGF5	E	T/C	0.29	0.46	-	8.5×10^{-25}	0.04	0.98	1.35	4.7×10^{-1}
4	rs223361	103769304	UBE2D3	E	T/C	0.66	0.17	0.02	2.7×10^{-20}	0.48	-0.10	0.54	8.5×10^{-1}
4	rs28667801	26785356	STIM2	E	A/T	0.59	-0.16	0.02	1.9×10^{-19}	0.87	-0.01	0.78	9.9×10^{-1}
4	rs55829085	2165493	POLN	E	A/C	0.95	-0.28	0.04	3.0×10^{-11}	0.99	2.13	2.50	4.0×10^{-1}
4	**rs7694000**	**95324968**	**PDLIM5**	**E**	**A/T**	**0.54**	**-0.10**	**0.02**	**3.5×10^{-8}**	**0.21**	**2.03**	**0.63**	**1.3×10^{-3}**
4	rs62312401	116987529	NDST4-TRAM1L1	AA	C/G	0.94	1.13	0.24	3.5×10^{-9}	0.04	-2.14	1.39	1.3×10^{-1}
5	rs12521868	131784393	C5orf56	E	T/G	0.37	-0.19	-	6.1×10^{-11}	0.04	-0.06	1.44	9.6×10^{-1}
5	rs1173771	32815028	NPR3-C5orf23	E	G/A	0.60	0.26	-	9.1×10^{-12}	0.82	1.04	0.72	1.5×10^{-1}
5	rs11953630	157845402	EBF1	E	T/C	0.37	-0.28	-	3.8×10^{-13}	0.13	1.27	0.77	9.9×10^{-2}
5	rs10077885	114390121	TRIM36	E	A/C	0.50	-0.17	0.03	4.0×10^{-11}	0.65	-0.40	0.55	4.7×10^{-1}
5	rs6891344	123136656	CSNK1G3	E	A/G	0.82	0.23	0.03	1.6×10^{-11}	0.72	0.45	0.56	4.1×10^{-1}
5	rs10078021	75038431	POC5	E	T/G	0.63	-0.16	0.03	1.3×10^{-8}	0.15	-0.41	0.69	5.5×10^{-1}
5	rs72812846	173377636	CPEB4	E	A/T	0.28	-0.21	0.03	2.2×10^{-11}	0.04	-0.18	1.43	9.0×10^{-1}
5	rs10062049	61553881	KIF2A	E	T/C	0.14	0.22	0.02	4.5×10^{-18}	0.32	0.62	0.63	3.2×10^{-1}
5	**rs954767**	**3706050**	**IRX1**	**E**	**A/C**	**0.74**	**-0.15**	**0.02**	**4.2×10^{-14}**	**0.68**	**1.14**	**0.54**	**3.6×10^{-2}**
5	rs55747751	132397351	HSPA4	E	A/G	0.08	-0.22	0.03	1.4×10^{-11}	0.01	-0.17	2.43	9.4×10^{-1}
5	**rs4286632**	**66291370**	**MAST4**	**E**	**A/G**	**0.73**	**0.12**	**0.02**	**1.9×10^{-9}**	**0.84**	**1.50***	**0.74**	**4.3×10^{-2}**
5	rs2188962	131770805	C5orf56	E/AA	T/C	0.37	-0.20	0.03	3.0×10^{-11}	0.03	1.25	1.70	4.6×10^{-1}
5	rs12515541	57095011	ACTBL2	E	T/G	0.61	0.12	0.02	6.2×10^{-11}	0.49	0.08	0.54	8.9×10^{-1}
6	rs926552	29548089	SNORD32B	E/AA	T/C	0.11	-0.26	0.05	7.2×10^{-8}	0.21	-0.25	0.63	7.0×10^{-1}
6	rs10943605	79655477	PHIP	E/AA	A/G	0.46	0.16	0.03	3.3×10^{-9}	0.28	0.10	0.57	8.6×10^{-1}

6	rs13205180	51832494	PKHD1	E	T/C	0.49	0.17	0.03	7.0×10^{-10}	0.12	0.43	0.81	6.0×10^{-1}
6	rs9372498	118572486	SLC35F1	E	A/T	0.08	0.33	0.05	1.8×10^{-11}	0.10	0.11	0.87	9.0×10^{-1}
6	rs147212971	166178451	PDE10A	E	T/C	0.06	-0.36	0.06	1.6×10^{-9}	0.17	0.34	0.74	6.5×10^{-1}
6	rs1799945	26091179	HFE	E	G/C	0.14	0.46	-	1.5×10^{-15}	0.02	-0.43	1.90	8.2×10^{-1}
6	**rs805303**	**31616366**	**BAT2-BAT5**	**E**	**G/A**	**0.61**	**0.23**	**-**	**3.0×10^{-11}**	**0.40**	**1.75***	**0.54**	**1.0×10^{-3}**
6	rs13209747	127115454	RSPO3	E/AA/AS	T/C	0.19	0.56	0.12	2.4×10^{-11}	0.07	-0.50	0.96	6.0×10^{-1}
6	rs17080102	151004770	PLEKHG1	E/AA/AS	C/G	0.10	-0.74	0.15	1.9×10^{-11}	0.15	-1.21	0.70	8.8×10^{-2}
6	rs9472135	43809802	VEGFA	E	T/C	0.70	0.15	0.02	4.3×10^{-16}	0.77	0.58	0.62	3.5×10^{-1}
6	rs668459	139835689	CITED2	E	T/C	0.59	-0.11	0.02	1.0×10^{-10}	0.28	0.02	0.57	9.7×10^{-1}
6	rs598682	154418759	OPRM1	E	A/G	0.25	-0.11	0.02	7.2×10^{-8}	0.03	0.23	1.31	8.6×10^{-1}
7	**rs17428471**	**27337867**	**EVX1-HOXA**	**E/AA/AS**	**T/G**	**0.14**	**0.61**	**0.14**	**1.6×10^{-9}**	**0.13**	**2.01***	**0.79**	**1.1×10^{-2}**
7	rs2969070	2512545	CHST12-LFNG	E	A/G	0.63	-0.18	0.03	2.9×10^{-11}	0.97	-0.24	1.58	8.8×10^{-1}
7	**rs11556924**	**129663496**	**ZC3HC1**	**E**	**T/C**	**0.38**	**-0.21**	**0.03**	**8.2×10^{-15}**	**0.01**	**6.90**	**2.72**	**1.2×10^{-2}**
7	rs6969780	27159136	HOXA3	E/AA	C/G	0.13	0.26	0.05	1.1×10^{-8}	0.39	0.68	0.54	2.1×10^{-1}
7	rs891511	150704843	NOS3	E/AA	A/G	0.37	-0.26	0.03	2.0×10^{-16}	0.59	-0.55	0.52	3.0×10^{-1}
7	rs1947228	96461649	SHFM1	E	T/C	0.42	-0.14	0.02	2.6×10^{-16}	0.96	1.01	1.29	4.4×10^{-1}
7	rs1722886	134215259	AKR1B10	E	A/T	0.57	0.12	0.02	3.8×10^{-12}	0.29	-0.05	0.57	9.3×10^{-1}
7	rs9638084	156311745	LINC01006	E	A/G	0.40	0.12	0.02	8.5×10^{-11}	0.55	-0.40	0.51	4.3×10^{-1}
7	rs11563582	27351650	EVX1-HOXA	AA	A/G	0.13	1.02	0.17	8.4×10^{-10}	0.17	0.80	0.71	2.6×10^{-1}
8	rs78192203	142375073	GPR20	AA	T/A	0.80	0.77	0.14	1.3×10^{-8}	0.79	-0.29	0.65	6.5×10^{-1}
8	rs2978098	101676675	SNX31	E	A/C	0.54	0.17	0.03	1.5×10^{-9}	0.03	-0.02	1.70	9.9×10^{-1}
8	rs62524579	144060955	P11–273G15.2	E	A/G	0.53	-0.18	0.03	3.8×10^{-9}	0.37	0.28	0.55	6.2×10^{-1}
8	rs10087782	141858620	PTK2	E	T/C	0.45	0.13	0.02	3.0×10^{-14}	0.82	0.32	0.67	6.3×10^{-1}
8	rs1047030	22428708	SORBS3	E	A/G	0.81	0.13	0.02	5.7×10^{-8}	0.96	-0.80	1.23	5.2×10^{-1}
9	rs4364717	21801530	MTAP	E	A/G	0.55	-0.18	0.03	1.3×10^{-10}	0.24	0.33	0.61	5.8×10^{-1}
9	rs76452347	35906471	HRCT1	E/AA	T/C	0.19	-0.23	0.04	6.8×10^{-10}	0.09	-0.16	0.92	8.6×10^{-1}
9	rs7020564	109670016	ZNF462	E	A/C	0.70	-0.11	0.02	6.7×10^{-9}	0.12	-0.54	0.82	5.1×10^{-1}
10	rs4746172	75855842	VCL	E	C/T	-	0.23	0.04	9.1×10^{-8}	0.23	-0.49	0.66	4.6×10^{-1}
10	rs1801253	115805056	ADRB1	E	G/C	0.27	-0.36	0.06	9.5×10^{-10}	0.34	-0.45	0.56	4.2×10^{-1}
10	rs10995311	64564934	ADO	E/AA	G/C	0.38	-0.20	0.03	2.1×10^{-11}	0.03	1.36	1.47	3.6×10^{-1}
10	rs1530440	63524591	C10orf107	E	T/C	0.19	-0.39	0.06	1.0×10^{-9}	0.03	-0.87	1.58	5.8×10^{-1}
10	rs4590817	63467553	C10orf107	E	G/C	0.84	0.42	-	1.3×10^{-12}	0.84	-0.81	0.68	2.4×10^{-1}
10	rs2782980	115781527	ADRB1	E	T/C	0.20	-0.28	0.05	9.6×10^{-8}	0.47	-0.76	0.53	1.5×10^{-1}
10	rs1813353	18707448	CACNB2	E	T/C	0.68	0.42	-	2.3×10^{-15}	0.84	0.16	0.73	8.3×10^{-1}
10	rs603424	102075479	PKD2L1	E	A/G	0.18	0.18	0.02	1.2×10^{-14}	0.75	0.36	0.63	5.7×10^{-1}
10	rs10906391	13523937	BEND7	E	T/C	0.32	0.13	0.02	7.6×10^{-12}	0.03	-0.27	1.65	8.6×10^{-1}
10	rs4373814	18419972	CACNB2(5′)	E	G/C	0.55	-0.22	-	4.4×10^{-10}	0.39	-0.35	0.53	5.1×10^{-1}

10	rs11191548	104846178	CYP17A1/NT5C2	E	T/C	0.91	0.46	-	9.4×10^{-13}	0.98	1.23	1.42	3.9×10^{-1}
11	rs4601790	65353906	EHBP1L1	E/AS	G/A	0.27	-0.02	0.04	9.9×10^{-9}	0.06	-0.32	1.05	7.6×10^{-1}
11	rs7103648	47461783	RAPSN-PSMC3-SLC39A13	E	A/G	0.61	-0.24	0.03	9.0×10^{-19}	0.85	0.78	0.74	3.0×10^{-1}
11	**rs751984**	**61278246**	**LRRC10B**	**E**	**T/C**	**0.88**	**0.38**	**0.04**	**4.2×10^{-20}**	**0.79**	**1.56***	**0.63**	**1.4×10^{-2}**
11	rs900145	13293905	ARNTL	E/AA	G/A	0.34	-0.20	0.03	1.8×10^{-8}	0.52	0.97	0.54	7.0×10^{-2}
11	rs11030119	27728102	BDNF	E	A/G	0.31	-0.16	0.03	2.9×10^{-8}	0.35	-0.36	0.54	5.1×10^{-1}
11	rs67330701	69079707	MYEOV	E	T/C	0.09	-0.37	0.05	2.1×10^{-12}	0.01	0.92	2.27	6.9×10^{-1}
11	**rs633185**	**100593538**	**FLJ32810-TMEM133**	**E**	**G/C**	**0.28**	**-0.33**	**-**	**2.0×10^{-15}**	**0.23**	**-1.49***	**6.23**	**1.7×10^{-2}**
11	rs381815	16902268	PLEKHA7	E	T/C	0.26	0.35	-	5.3×10^{-14}	0.26	0.58	0.62	3.4×10^{-1}
11	rs1401454	16250183	SOX6	E/AA/AS	T/C	0.46	0.45	0.10	5.1×10^{-10}	0.46	-0.27	0.52	6.1×10^{-1}
11	rs360153	9762274	SWAP70	E	T/C	0.42	-0.22	0.02	4.4×10^{-36}	0.52	-0.30	0.52	5.7×10^{-1}
11	rs11026586	22515533	RP11-34N19.1	E	A/G	0.07	0.29	0.03	2.7×10^{-17}	0.06	0.43	1.21	7.3×10^{-1}
11	rs875106	70005641	ANO1	E	A/G	0.52	-0.13	0.02	1.7×10^{-14}	0.71	1.02	5.8	7.7×10^{-2}
11	rs4420291	74374950	POLD3	E	A/G	0.51	0.10	0.02	2.2×10^{-8}	0.33	0.08	0.58	8.9×10^{-1}
11	**rs7129220**	**10350538**	**ADM**	**E**	**G/A**	**0.89**	**-0.30**	**-**	**6.4×10^{-8}**	**0.92**	**-0.79***	**1.01**	**4.4×10^{-3}**
12	rs17249754	90060586	ATP2B1	E	G/A	0.84	0.52	-	1.2×10^{-18}	0.85	-1.89	0.69	7.0×10^{-1}
12	rs10850411	115387796	TBX5/TBX3	E	T/C	0.70	0.25	-	5.4×10^{-10}	0.61	0.32	0.53	5.5×10^{-1}
12	rs1060105	123806219	SBNO1	E/AS	T/C	0.21	-0.18	-	3.1×10^{-8}	0.05	-1.08	1.22	3.8×10^{-1}
12	rs2384550	115352731	TBX5-TBX3	E	A/G	0.35	-0.35	0.06	3.7×10^{-8}	0.35	-0.93	0.53	7.9×10^{-2}
12	rs35444	115552437	TBX3	AS	A/G	0.75	0.50	0.05	1.3×10^{-10}	0.56	-0.66	0.53	2.1×10^{-1}
12	rs7302981	50537815	CERS5	E/AA	A/G	0.34	0.25	0.03	9.4×10^{-19}	0.10	0.90	0.84	2.8×10^{-1}
12	rs7132012	8832203	RP11-20D14.4	E	A/G	0.68	0.15	0.02	3.2×10^{-17}	0.57	0.08	0.51	8.8×10^{-1}
12	rs1271309	124820705	NCOR2	E	A/G	0.16	-0.20	0.02	1.5×10^{-16}	0.08	0.22	0.92	8.1×10^{-1}
12	rs7137749	57098040	NACA	E	T/C	0.37	0.14	0.02	7.2×10^{-15}	0.52	-0.44	0.52	4.1×10^{-1}
12	rs7134060	96717095	CDK17	E	A/G	0.45	-0.11	0.02	1.1×10^{-9}	0.26	-0.93	0.60	1.2×10^{-1}
12	rs75507123	5417856	RP11-1038A11.3	E	T/G	0.13	-0.14	0.03	3.9×10^{-8}	0.02	-0.66	1.70	7.0×10^{-1}
12	rs1098708	27321112	STK38L	E	A/G	0.28	-0.10	0.02	4.6×10^{-8}	0.82	1.08	0.67	1.1×10^{-1}
13	rs55684003	97988689	MBNL2	E	A/G	0.70	0.12	0.02	1.0×10^{-10}	0.95	-1.83	1.14	1.1×10^{-1}
13	rs9563529	58316637	PCDH17	E	T/G	0.21	0.12	0.02	1.4×10^{-8}	0.26	0.56	0.59	3.4×10^{-1}
14	rs11628933	60700903	PPM1A	E	C/G	0.23	-0.12	0.02	3.1×10^{-9}	0.40	-0.41	0.54	4.5×10^{-1}
14	rs4424827	35110857	SNX6	E	T/C	0.57	-0.10	0.02	2.1×10^{-8}	0.94	-0.38	7.20	9.6×10^{-1}
15	**rs7178615**	**66869072**	**RP11–321F6.1**	**E**	**A/G**	**0.37**	**-0.18**	**0.03**	**2.6×10^{-10}**	**0.19**	**1.42**	**0.69**	**4.1×10^{-2}**
15	rs62012628	79070000	ADAMTS7	E	T/C	0.29	-0.24	0.03	5.1×10^{-12}	0.31	-0.03	0.58	9.6×10^{-1}
15	rs12906962	95312071	chr15mb95	E	T/C	0.68	-0.22	0.03	5.6×10^{-14}	0.21	-0.73	0.64	2.5×10^{-1}
15	**rs1378942**	**75077367**	**CYP1A1-ULK3**	**E**	**C/A**	**0.36**	**0.48**	**0.09**	**6.0×10^{-8}**	**0.97**	**2.99***	**1.48**	**4.4×10^{-2}**
15	rs2521501	91437388	FURIN-FES	E	T/A	0.31	0.36	-	1.9×10^{-15}	0.21	-1.11	0.66	9.5×10^{-2}
15	rs873122	92702020	SLCO3A1	E	C/G	0.72	0.12	0.02	6.5×10^{-10}	0.94	2.00	1.16	8.5×10^{-2}

15	rs7180952	85162551	ZSCAN2	E	T/C	0.54	-0.10	0.02	9.8×10^{-9}	0.75	0.02	0.61	9.8×10^{-1}
15	**rs62004794**	**68454523**	**PIAS1**	**E**	**A/G**	**0.44**	**-0.10**	**0.02**	**3.4×10^{-8}**	**0.60**	**-1.16***	**0.55**	**3.6×10^{-2}**
16	rs12921187	4943019	PPL	E	T/G	0.43	-0.17	0.03	2.5×10^{-10}	0.96	-2.36	1.23	5.5×10^{-2}
16	rs72799341	30936743	FBXL19	E	A/G	0.24	0.19	0.03	5.8×10^{-9}	0.09	0.03	0.90	9.8×10^{-1}
16	rs8059962	81574197	CMIP	E	T/C	0.42	-0.17	0.03	1.3×10^{-9}	0.61	0.37	0.52	4.8×10^{-1}
16	rs1126464	89704365	DPEPI	E/AA	C/G	0.22	0.24	0.03	2.4×10^{-13}	0.06	-0.90	1.10	4.2×10^{-1}
16	rs45474499	66914492	PDP2	E	T/C	0.05	0.36	0.04	8.5×10^{-18}	0.07	0.85	1.09	4.3×10^{-1}
16	rs7185555	69131281	HAS3	E	C/G	0.15	-0.15	0.02	2.3×10^{-10}	0.31	0.42	0.56	4.6×10^{-1}
16	**rs9932866**	**706067**	**WDR90**	**E**	**A/G**	**0.37**	**0.12**	**0.02**	**2.9×10^{-10}**	**0.90**	**-1.61**	**0.80**	**4.5×10^{-2}**
17	rs4308	61559625	ACE	E	A/G	0.37	0.21	0.03	6.8×10^{-14}	0.03	-1.29	1.73	4.5×10^{-1}
17	rs12940887	47402807	ZNF652	E	T/C	0.38	0.27	-	2.3×10^{-14}	0.07	0.41	1.25	7.4×10^{-1}
18	rs12958173	42141977	SETBP1	E	A/C	0.31	0.18	0.03	5.8×10^{-10}	0.31	0.68	0.56	2.2×10^{-1}
18	rs745821	48142854	MAPK4	E	T/G	0.76	0.19	0.03	1.4×10^{-9}	0.70	0.21	0.58	7.1×10^{-1}
18	rs34163044	51851616	STARD6	E	A/C	0.42	0.15	0.02	9.6×10^{-17}	0.31	-0.13	0.59	8.3×10^{-1}
18	rs11665020	10879503	PIEZO2	E	C/G	0.32	-0.14	0.02	2.8×10^{-14}	0.14	-0.18	0.72	8.1×10^{-1}
18	rs4800420	20158965	CTAGE1	E	A/G	0.29	0.12	0.02	5.2×10^{-10}	0.17	-0.63	0.70	3.7×10^{-1}
19	rs167479	11526765	RGL3	E/AA	T/G	0.45	-0.30	0.03	4.2×10^{-28}	0.17	-0.23	0.71	7.4×10^{-1}
19	rs62104477	30294991	CCNE1	E	T/G	0.33	0.18	0.03	1.2×10^{-9}	0.25	0.47	0.61	4.4×10^{-1}
19	rs4247374	7252756	INSR	E	T/C	0.14	-0.39	0.03	2.1×10^{-22}	0.02	-1.85	1.96	3.5×10^{-1}
19	rs2304130	19789528	ZNF101	E/AS	A/G	0.91	-0.29	-	2.0×10^{-8}	0.74	-0.63	0.59	2.9×10^{-1}
19	rs9710247	40760449	AKT2	E	A/G	0.45	0.16	0.03	1.6×10^{-9}	0.90	-1.29	0.92	1.6×10^{-1}
19	rs1821295	32590773	AC011518.1	E	T/C	0.70	-0.14	0.02	3.1×10^{-13}	0.92	-0.06	1.14	9.6×10^{-1}
20	rs6095241	47308798	PREX1	E/AS	A/G	0.45	-0.17	-	4.8×10^{-9}	0.60	-0.42	0.53	4.4×10^{-1}
20	rs6108168	8626271	PLCB1	E	A/C	0.25	-0.21	0.03	1.1×10^{-11}	0.62	-0.08	0.53	8.8×10^{-1}
20	rs1327235	10969030	JAG1	E	G/A	0.46	0.30	-	1.4×10^{-15}	0.59	-0.09	0.52	8.6×10^{-1}
20	rs6015450	57751117	GNAS-EDN3	E	G/A	0.12	0.56	-	5.6×10^{-23}	0.18	0.74	0.65	2.6×10^{-1}
20	rs1232482	11886643	BTBD3	E	T/C	0.40	-0.12	0.02	6.1×10^{-12}	0.18	-1.26	0.70	7.3×10^{-2}
21	**rs12627651**	**44760603**	**CRYAA-SIK1**	**E**	**A/G**	**0.29**	**0.20**	**0.03**	**1.4×10^{-11}**	**0.07**	**2.55***	**1.12**	**2.3×10^{-2}**

Chr; chromosomes, SNP; single nucleotide polymorphism, E; European ancestry, AA; African ancestry, AS; Asian ancestry, EA; effect allele, RA; reference allele, EAF; effect allele frequency, β; Effect size estimates correspond to mean difference in mmHg per effect allele for systolic or diastolic blood pressure, adjusted for age and body mass index, s.e; standard error

[†] Given with respect to Build 37 (GRCh37/hg19)

[‡] Adjusted for age and body mass index

Bold indicate p-value $<5.0 \times 10^{-2}$ for replication analysis

* Indicate same β direction in both the discovery and EMaBS populations

The observed P-values show no departure from the null (Figure 7.2-2), either for systolic or diastolic BP, with lambda values of 1.006 and 0.995, respectively. The results show adequate control for population substructure in the analysis.

The SNPs most strongly associated with BP are shown in Table 1. None of the top scoring SNPs reached genome-wide levels of significance (p-value $<5x10^{-8}$) for association with adolescent systolic or diastolic BP. Borderline significance ($5x10^{-8} <$ p-value $<1x10^{-6}$) for association with systolic BP was achieved for 4 index SNPs at 4 separate loci; there were 4 index SNPs showing borderline significant associations with diastolic BP, at 4 separate loci. There was no overlap between the SNPs most strongly associated with systolic BP and those most strongly associated with diastolic BP. None of these most strongly associated SNPs have been identified as associated with BP in previously published BP GWAS. The most strongly associated SNP for systolic BP with p-value $6.8x10^{-7}$ was rs181430167, located in the intron region of the *KLHL29* gene on chromosome 2. The lowest p-value ($4.0x10^{-7}$) for association with diastolic BP was for rs12991132 located between *ZFP36L2, THADA, LOC1001297261* genes on chromosome 2.

Of the 389 SNPs [1, 2, 5, 6, 13-18, 34-53] identified for replication, 330 (85%) SNPs were included in the replication analysis. Fifty-nine SNPs that were either rare (<0.01) or poorly imputed (INFO score <0.3) in the EMaBS sample were not included in the replication analysis. Thirty SNPs of the 330 SNPs included in the replication had been previously associated with BP in populations of African origins. Forty SNPs had been previously associated with both systolic and diastolic BP and were tested for association with both. Tables 2 and 3 show results from the replication analysis. Briefly, 33 SNPs (17 for systolic, 15 for diastolic and one for both systolic and diastolic BP) were associated with BP in this population, with the same effect direction as the discovery population for 14 of the SNPs (5 for systolic BP and 8 for diastolic BP and one for both systolic and diastolic BP).

Of the 30 SNPs previously known to be associated with BP specifically in individuals of African origin, three (*ATP2B* rs2681492, *MDM4* rs2169137 and *EVX1*/*HOXA* rs17428471) were associated with BP in the present study. Only the *EVX1*/*HOXA* rs17428471 had the same effect direction as in the discovery population. The *BAT2*/*BAT5* rs805303 variant was associated with systolic BP and diastolic BP of the 40 SNPs tested for association with both traits. The G allele of the *BAT2*/*BAT5* rs805303 variant was associated with higher systolic and higher diastolic BP among adolescents in this study: the same effect direction observed in the discovery population for both traits.

There were 370 independent tests (197 for systolic BP, 173 for diastolic BP) conducted, thus approximately 19 SNPs (370 x 0.05=18.5) would be expected to be associated with BP at p-value <0.05 by chance alone. None of the replicated SNPs met a Bonferroni corrected significance threshold ($0.05/370=1.35x10^{-4}$), although one (rs6712094 intergenic between *FIGN* and *GRB14*) was very close (p-value=$2.6x10^{-4}$) for association with systolic BP. The most strongly associated SNPs for association with diastolic BP were rs805303 between *BAT2* and *BAT5* (p-value=$1.0x10^{-3}$) and *PDLIM5* rs7694000 (p-value=$1.3x10^{-3}$).

Discussion

To our knowledge, this is the first genetic analysis examining variants associated with BP among African adolescents. We hypothesized that common genetic variants (unique or not unique to populations in Africa) were associated with systolic and, or diastolic BP in Ugandan adolescents and that these associations may overlap with associated variants identified in previous studies of Africans. The GWAS revealed no novel or previously identified variant associated with systolic or diastolic BP in our study population. Thirty-three SNPs were associated with BP in the replication analysis, with the direction of effect consistent with the discovery population for 14 SNPs. There were no SNPs reaching a Bonferroni-adjusted significance level. None of the replicated SNPs were located in genes with monogenic effect on hypertension [23].

The SNPs most strongly associated with either systolic or diastolic BP were of borderline significance and none has been reported as associated with BP in previous BP GWASs. It is possible that the most strongly associated SNPs are in LD with the BP causing variants. The most strongly associated SNPs were mostly common variants with modest effect sizes and might uniquely influence BP in African population. It is important for larger genetic studies of African population to investigate the role of these SNPs in BP regulation among Africans. These tops SNPs are potential candidates for replication analysis in African populations.

The failure to identify variants strongly associated with BP presumably occurred because the study was underpowered to detect effects of rare variants or small effects of common variants. Blood pressure is most likely a polygenic trait influenced by the simultaneous presence of several gene variants each with a small effect size and contributing in an additive manner to BP expression. Thus, the large effect sizes that this study had good power to detect, may not be realistic. For example, the present study had 80% power to detect a 3.2 mmHg change in mean systolic BP for a minor allele frequency of 20% at

genome-wide significance level, p-value $<5x10^{-8}$. Many of the variants reliably associated with BP in adults have an effect size of 0.5mmHg or less [6]. In addition to the limitation caused by the relatively small sample size, imputation did not allow inference for rare variants not included in the imputation SNP panel.

Few GWAS "top SNPs" from non-African populations have been replicated in populations of African ancestry [15, 16, 19]. Variants associated with BP in populations of African origin might be different from variants that influence BP in Caucasian populations or not in LD with the BP causing variants. Our replication study was limited to variants associated with BP from previous GWAS of BP in other populations. Thirty-three SNPs identified from previous BP GWAS were replicated, most of these were previously identified in populations of non-African origins. Of the identified loci, the *PAX2* gene is essential in the development of the renal epithelium [54] and plays a critical role in kidney development [55]. The kidneys are critical in BP regulation. Two of the replicated SNPs are located on the *ATP2B1* gene. The *ATP2B1* gene is involved in calcium homeostasis [56]. The *ATP2B1* rs2681492, *MDM4* rs2169137, *EVX1*/*HOXA* rs17428471 SNPs previously associated with BP in trans-ethnic populations (African, Caucasian and Asian) were associated with adolescent BP in the present study. These genes most likely influence BP across different ethnic groups.

Replication studies in diverse populations have returned mixed results. This current study conducted 370 tests using 330 SNPs, of which 33 SNPs (one SNP for both traits) were associated with BP. Failure to replicate most variants associated with BP in other populations could be due to differences in minor allele frequencies across populations or differences in LD patterns combined with a poor understanding of the causative variants or due to spurious initial findings. Blood pressure is likely to be influenced by the simultaneous presence of several genetic variants, each conferring a small change in BP.

Although none of the variants reached Bonferroni levels of significance more associated variants were identified than expected under the null suggesting that some of these variants found to be associated could be worthy of further follow-up. Fourteen variants more than those expected by chance (19 variants) under the null hypothesis were associated with BP in this study.

Of the 40 SNPs tested for association with both traits, only *BAT2*/*BAT5* rs805303 was associated with both traits in the adolescents, suggests that not many genetic loci have influence on both systolic and diastolic BP. Similar to an earlier replication study among adult Ugandans [19], the *ATP2B1* rs2681492 was associated with BP in these Ugandan

adolescents, but with an opposite effect direction to the discovery population [37] and Ugandan adults [19]. The G allele of the *ATP2B1* rs2681492 was associated with lower systolic BP in Ugandans adults [19] but with higher systolic BP in the present study.

Some loci may have varying roles in BP regulation across different populations. A European study investigated SNPs associated with BP at different age epochs (using independent samples for each age group): prepuberty (age 4-7 years), pubertal (8-12 years) and post-pubertal (13-20 years). The A allele of *TGA11* rs1563894 was associated with lower systolic BP in prepuberty while the T allele of *SMARCA2*/*VLDLR* rs872256 was associated with higher systolic during puberty [48]. No SNP was associated with BP in the post-pubertal period, and no SNP was consistently associated with BP across all three age groups. The *TGA11* rs1563894 and *SMARCA2*/*VLDLR* rs872256 were not replicated in this present study.

The present study has several strengths. This is the first BP GWAS of an African population and the first candidate gene analysis among adolescents residing in Africa. Participants in this study were similar to non-participants with respect to most baseline characteristics. Rigorous quality control procedures were used during the measurement of the various variables including BP and genotyping. Data from this study can contribute to future BP GWAS meta-analyses. A key limitation of this study was that it was under-powered to detect effects of rare variants and also to enable testing for effect modification by sex and other environmental variables.

Future work should take advantage of various African cohorts to form consortia that can enable the conduct of GWAS meta-analysis well powered to identify rare and low-frequency variants that may be associated with BP in African populations. Future candidate gene analysis using a sample from a different geographical region or ethnic background should investigate for interactions between variants, this might help our understanding of the aetiology of BP. It is possible that multiple interacting variants (rare and common) are influencing BP levels in this population. Although we did not formally allow for multiple testing in the replication analysis, the current study had 33 associated SNPs 14 more than expected by chance. Polygenic scores analysis of variants associated with BP among African populations may explain the missing BP heritability.

In summary, we conducted the first genetic study of BP phenotypes among Ugandan adolescents. Although this study did not identify novel BP variants, replication of some previously identified variants suggests that some genetic variants may universally

influence BP susceptibility. Large scale studies in African populations are required to identify novel and evaluate previously reported loci.

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Conflict of interest

The authors declare that they have no conflict of interest

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Reference

1. Warren, H.R., et al., Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. Nat Genet, 2017. **49**(3): p. 403-415.
2. Levy, D., et al., Genome-wide association study of blood pressure and hypertension. Nat Genet, 2009. **41**(6): p. 677-87.
3. Xi, B., et al., Association of common variants in/near six genes (ATP2B1, CSK, MTHFR, CYP17A1, STK39 and FGF5) with blood pressure/hypertension risk in Chinese children. J Hum Hypertens, 2014. **28**(1): p. 32-6.
4. Qian, X., et al., A meta-analysis of association between C677T polymorphism in the methylenetetrahydrofolate reductase gene and hypertension. Eur J Hum Genet, 2007. **15**(12): p. 1239-45.
5. Cho, Y.S., et al., A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. Nat Genet, 2009. **41**(5): p. 527-34.
6. Evangelou, E., et al., Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. Nat Genet, 2018. **50**(10): p. 1412-1425.
7. Noubiap, J.J., et al., Prevalence of elevated blood pressure in children and adolescents in Africa: a systematic review and meta-analysis. The Lancet Public Health, 2017. **2**(8): p. e375-e386.
8. Addo, J., L. Smeeth, and D.A. Leon, Hypertension in sub-saharan Africa: a systematic review. Hypertension, 2007. **50**(6): p. 1012-8.
9. Tishkoff, S.A., et al., The genetic structure and history of Africans and African Americans. Science, 2009. **324**(5930): p. 1035-44.
10. Luoni, G., et al., Population-specific patterns of linkage disequilibrium in the human 5q31 region. Genes Immun, 2005. **6**(8): p. 723-7.
11. Peprah, E., et al., Genome-wide association studies in Africans and African Americans: expanding the framework of the genomics of human traits and disease. Public Health Genomics, 2015. **18**(1): p. 40-51.

12. Yako, Y.Y., et al., Genetic factors contributing to hypertension in African-based populations: A systematic review and meta-analysis. J Clin Hypertens (Greenwich), 2018. **20**(3): p. 485-495.

13. Fox, E.R., et al., Association of genetic variation with systolic and diastolic blood pressure among African Americans: the Candidate Gene Association Resource study. Hum Mol Genet, 2011. **20**(11): p. 2273-84.

14. Liang, J., et al., Single-trait and multi-trait genome-wide association analyses identify novel loci for blood pressure in African-ancestry populations. PLoS Genet, 2017. **13**(5): p. e1006728.

15. Adeyemo, A., et al., A genome-wide association study of hypertension and blood pressure in African Americans. PLoS Genet, 2009. **5**(7): p. e1000564.

16. Franceschini, N., et al., Genome-wide association analysis of blood-pressure traits in African-ancestry individuals reveals common associated genes in African and non-African populations. Am J Hum Genet, 2013. **93**(3): p. 545-54.

17. Kidambi, S., et al., Non-replication study of a genome-wide association study for hypertension and blood pressure in African Americans. BMC Med Genet, 2012. **13**: p. 27.

18. Li, C., et al., Genome-Wide Association Study Meta-Analysis of Long-Term Average Blood Pressure in East Asians. Circ Cardiovasc Genet, 2017. **10**(2): p. e001527.

19. Kayima, J., et al., Association of genetic variation with blood pressure traits among East Africans. Clin Genet, 2017. **92**(5): p. 487-494.

20. Williams, R.R., et al., Are there interactions and relations between genetic and environmental factors predisposing to high blood pressure? Hypertension, 1991. **18**(3 Suppl): p. I29-37.

21. Biron, P., J.G. Mongeau, and D. Bertrand, Familial aggregation of blood pressure in 558 adopted children. Can Med Assoc J, 1976. **115**(8): p. 773-4.

22. Salfati, E., et al., Direct Estimates of the Genomic Contributions to Blood Pressure Heritability within a Population-Based Cohort (ARIC). PLoS One, 2015. **10**(7): p. e0133031.

23. Ehret, G.B. and M.J. Caulfield, Genes for blood pressure: an opportunity to understand hypertension. Eur Heart J, 2013. **34**(13): p. 951-61.

24. Manolio, T.A., et al., Finding the missing heritability of complex diseases. Nature, 2009. **461**(7265): p. 747-53.

25. Maher, B., Personal genomes: The case of the missing heritability. Nature, 2008. **456**(7218): p. 18-21.

26. Elliott, A.M., et al., The impact of helminths on the response to immunization and on the incidence of infection and disease in childhood in Uganda: design of a randomized, double-blind, placebo-controlled, factorial trial of deworming interventions delivered in pregnancy and early childhood [ISRCTN32849447]. Clin Trials, 2007. **4**(1): p. 42-57.

27. Lule, S.A., et al., Are birthweight and postnatal weight gain in childhood associated with blood pressure in early adolescence? Results from a Ugandan birth cohort. Int J Epidemiol, 2018.

28. Purcell, S., et al., PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet, 2007. **81**(3): p. 559-75.

29. Genomes Project, C., et al., A global reference for human genetic variation. Nature, 2015. **526**(7571): p. 68-74.

30. Gurdasani, D., et al., The African Genome Variation Project shapes medical genetics in Africa. Nature, 2015. **517**(7534): p. 327-32.

31. O'Connell, J., et al., A general approach for haplotype phasing across the full spectrum of relatedness. PLoS Genet, 2014. **10**(4): p. e1004234.

32. Marchini, J., et al., A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet, 2007. **39**(7): p. 906-13.

33. Yang, J., et al., GCTA: a tool for genome-wide complex trait analysis. Am J Hum Genet, 2011. **88**(1): p. 76-82.

34. International Consortium for Blood Pressure Genome-Wide Association, S., et al., Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature, 2011. **478**(7367): p. 103-9.

35. Liu, C., et al., Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci. Nat Genet, 2016. **48**(10): p. 1162-70.

36. Newton-Cheh, C., et al., Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet, 2009. **41**(6): p. 666-76.

37. Hoffmann, T.J., et al., Genome-wide association analyses using electronic health records identify new loci influencing blood pressure variation. Nat Genet, 2017. **49**(1): p. 54-64.

38. Ganesh, S.K., et al., Loci influencing blood pressure identified using a cardiovascular gene-centric array. Hum Mol Genet, 2013. **22**(8): p. 1663-78.

39. Tragante, V., et al., Gene-centric meta-analysis in 87,736 individuals of European ancestry identifies multiple blood-pressure-related loci. Am J Hum Genet, 2014. **94**(3): p. 349-60.

40. Simino, J., et al., Gene-age interactions in blood pressure regulation: a large-scale investigation with the CHARGE, Global BPgen, and ICBP Consortia. Am J Hum Genet, 2014. **95**(1): p. 24-38.

41. Surendran, P., et al., Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension. Nat Genet, 2016. **48**(10): p. 1151-1161.

42. Ehret, G.B., et al., The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. Nat Genet, 2016. **48**(10): p. 1171-1184.

43. Kato, N., et al., Trans-ancestry genome-wide association study identifies 12 genetic loci influencing blood pressure and implicates a role for DNA methylation. Nat Genet, 2015. **47**(11): p. 1282-1293.

44. Johnson, T., et al., Blood pressure loci identified with a gene-centric array. Am J Hum Genet, 2011. **89**(6): p. 688-700.

45. Ho, J.E., et al., Discovery and replication of novel blood pressure genetic loci in the Women's Genome Health Study. J Hypertens, 2011. **29**(1): p. 62-9.

46. Takeuchi, F., et al., Blood pressure and hypertension are associated with 7 loci in the Japanese population. Circulation, 2010. **121**(21): p. 2302-9.

47. Newton-Cheh, C., et al., Association of common variants in NPPA and NPPB with circulating natriuretic peptides and blood pressure. Nat Genet, 2009. **41**(3): p. 348-53.

48. Parmar, P.G., et al., International Genome-Wide Association Study Consortium Identifies Novel Loci Associated With Blood Pressure in Children and Adolescents. Circ Cardiovasc Genet, 2016. **9**(3): p. 266-78.

49. Wain, L.V., et al., Novel Blood Pressure Locus and Gene Discovery Using Genome-Wide Association Study and Expression Data Sets From Blood and the Kidney. Hypertension, 2017.

50. Wain, L.V., et al., Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. Nat Genet, 2011. **43**(10): p. 1005-11.

51. Ganesh, S.K., et al., Effects of long-term averaging of quantitative blood pressure traits on the detection of genetic associations. Am J Hum Genet, 2014. **95**(1): p. 49-65.

52. Johnson, A.D., et al., Association of hypertension drug target genes with blood pressure and hypertension in 86,588 individuals. Hypertension, 2011. **57**(5): p. 903-10.

53. Kato, N., et al., Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in east Asians. Nat Genet, 2011. **43**(6): p. 531-8.

54. Dressler, G.R. and A.S. Woolf, Pax2 in development and renal disease. Int J Dev Biol, 1999. **43**(5): p. 463-8.

55. Hou, X.M., X. Chen, and Y.L. Wang, [The role of Pax2 in regulation of kidney development and kidney disease]. Yi Chuan, 2011. **33**(9): p. 931-8.

56. Hirawa, N., A. Fujiwara, and S. Umemura, ATP2B1 and blood pressure: from associations to pathophysiology. Curr Opin Nephrol Hypertens, 2013. **22**(2): p. 177-84.

7.3 Supplementary tables

Table S1: Characteristics of the Entebbe Mother and Baby Study participants included and not included in the genetic analysis study (N=2345)

Mothers' characteristics during pregnancy	Participated (n=815)		Did not participate (n=1530)	
	Number	Percentage/ Mean (SD)	Number	Percentage/ Mean (SD)
Age (years)*	815	24.5 (5.6)	1530	23.3 (5.2)
Parity (number of children) *	815	3.0 (1.8)	1530	2.7 (1.7)
Body mass index*	809	24.0 (3.2)	1509	24.1 (3.2)
Household SES index (1 lowest, 6 highest)	806	3.8 (1.2)	1495	3.6 (1.2)
Education level				
None	20	2.5	66	4.3
Primary	392	48.2	800	52.4
Senior	317	39.0	554	36.3
Tertiary	84	10.3	108	7.1
Marital status				
Single	85	10.4	222	14.5
Married/cohabiting	709	87.1	1257	82.2
Separated/widowed	20	2.5	51	3.3
Area of residence				
Urban	574	71.3	1027	68.1
Rural	231	28.7	481	31.9
Infections				
Asymptomatic malaria	75	9.4	173	11.5
Schistosomiasis	134	16.5	287	18.8
Hookworm	322	39.6	703	46.2
Ascaris	21	2.6	33	2.2
Trichuris	67	8.2	139	9.1
Trichostrongylus	8	1.0	14	0.9
Participants' characteristics				
Birthweight (Kg)*	692	3.2 (0.5)	1204	3.2 (0.5)
Sex				
Male	417	51.1	794	52.0
Birth type				
Singleton	802	98.4	1488	97.3
Multiple	13	1.6	42	2.8
Feeding at 6 weeks of age				
Exclusive breastfeeding	579	71.5	893	63.6
Mixed feeding	219	27.0	502	35.8
Weaned	12	1.8	9	0.6
HIV status				
Unexposed	731	89.7	1334	87.2
Exposed not infected	76	9.3	150	9.8
Infected	8	1.0	46	3.0
Place of birth				
Entebbe Hospital	611	75.0	1069	70.3
Home	85	10.4	179	11.8
Others	119	14.6	273	18.0
Mode of delivery				
Normal	731	89.7	1385	91.0
Caesarean section	77	9.5	120	7.9
Instrumentation	7	0.9	17	1.2

* Mean value with standard deviation (SD) presented
Percentages may total to ± 100 due to rounding
Household socioeconomic status (SES) was a composite variable and could take values 1 (low) to 6 (high)
Missing data for (a) mother's characteristics: body mass index 27; household SES 44; education 4; marital status 1; place of residence 32; asymptomatic malaria 43; schistosomiasis 9; hookworm 9; ascaris 9; trichuris 9; trichostrongylus 9; (b) child's characteristics: sex 2; birth weight 449; feeding status 131; place of delivery 9; mode of delivery 8

Chapter 8: Discussion

8.1 Preface

This chapter discusses the main findings of the thesis, implications for research and policy, strengths and limitations, and generalisability of findings. This PhD comprised of a systematic review of literature published on the relationship between birth weight and BP among young Africans, and an observational study using prospectively collected data from participants born in the EMaBS, a tropical birth cohort of 2,345 live born infants. The observational study was designed to investigate the effect of birth weight, postnatal growth, pre- and peri-natal and childhood exposures and of genetic polymorphisms on adolescents' BP. Respectively, we hypothesised that birth weight (a proxy for intra-uterine growth) and change in WAZ (a proxy for postnatal growth) were negatively and positively associated with BP in early adolescents. We hypothesised that several environmental and genetic factors, including those more common in Africa than in other settings would also be associated with BP in adolescents.

8.2 Main findings

8.2.1 Systematic review

As previously reported in chapter 3, Medline, EMBASE, Global Health and Web of Science databases were systematically searched for relevant publications up to October 2016, on the relationship between birth weight and BP among young Africans aged 0 to 18 years. Reviewed studies varied in size (from 157 to 2,743 participants per study), were either cross-sectional or cohort in design and either hospital- or community-based [1].

The systematic review showed that the relationship between birth weight and BP among young Africans remains under-studied, with only 16 relevant papers (from 13 study groups in nine African countries) identified. Evidence from this small number of publications (studies) suggested that the relationship between birth weight and BP varied with participants' age; a consistently positive association was seen in neonates (age 0-28 days), predominantly inverse associations seen in children (age 1-9 years) and inverse or no association seen in adolescents (age 10-18 years) [1].

A possible explanation for the observed positive association between birth weight and BP in neonates is that, the short duration between birth and BP assessment does not allow for any impact of subsequent growth trajectory on BP to develop. The effect of the measured birth weight may not reflect the effect of birth weight but the effect of current weight on

BP in the neonates. Current weight at birth, which in neonates is birth weight, is reported to be associated with BP. Thus, in the neonates the impact of growth trajectory is yet to be felt.

The inverse association between birth weight and BP among African children in this current review, supports earlier findings in children from Africa and other settings reviewed in [2-4]. The findings in children strengthen the fetal origin hypothesis, that low birth weight predicts high BP later in life, first published close to three decades ago by Barker et al [5].

The inconsistent (inverse or no) association between birth weight and BP in adolescents reviewed in the present study also supports findings in earlier reviews. Inconsistencies in the birth weight-BP relationships observed in adolescents may partly be due to rapid pubertal growth due to hormonal changes which start at varied age during adolescence. Pubertal growth is associated with greater increases in BP than growth in pre- and post-pubertal periods [6, 7].

The discrepancies in the birth weight-BP relationship in young Africans were less likely to be due to differences in study procedures as similar approaches were followed: a rest period before and between successive BP measurements; automated devices were used; the mean of three or two BP measurements was used for most analyses; and birth weight data were prospectively collected nearly in all studies. The small number of studies from Africa on the birth weight-BP relationship prohibit definitive conclusions to be made.

An additional search for manuscripts published after 15th October 2016, identified one relevant study on the relationship between birth weight and BP. This study among 528 rural South Africans aged 5 to 15 years, showed that birth weight was not associated with BP [8].

In summary, the small number of preceding studies from Africa justified the observational study using longitudinally collected prospective data from 2,345 mother-child pairs in the EMaBS birth cohort.

8.2.2 Observational study

For the first time in mainland East Africa, we aimed to describe the effect of early-life (fetal and postnatal) growth, life-course factors and genetic variants on BP among early adolescents aged 10 and 11 years. In the EMaBS, data was collected prenatally from 2,507 women and their resulting 2,345 live born offspring were followed from birth to 10 or 11years of age. From 20th May 2014 to 16th June 2016 additional data including BP

measurements, anthropometry, physical activity, and diet were obtained from 1,119 participants. Genetic data from 1,391 participants had been earlier generated using stored samples obtained at one year of age.

8.2.2.1 High blood pressure and pre-hypertension prevalence

The prevalence of high BP and pre-hypertension was 8% and 11% respectively on day-one among 10- and 11-year-old adolescents. The prevalence of hypertension after measurements taken on three separate occasions was low, estimated at 1%. Only those with average systolic and/or diastolic BP ≥95th percentile for sex, age and height on the first occasion were invited for extra BP measurements on the second and third occasion, and this may have biased the estimate.

Our estimates of high BP and hypertension prevalence were lower than earlier reported in 8- to 18-year-old students from the same study area [9]. However, the estimate of high BP prevalence was comparable to that among 5- to 16-year-olds from the Seychelles [10] and in 10- to 17-year-old Gambians [11], but higher than in 12- to 18-year-old Nigerians [12].

8.2.2.2 Early growth and adolescent BP

Birth weight was not associated with BP, although at crude analysis, there was some suggestion of a U- or J-shaped relationship between birth weight and BP. The present study shows that rapid increases in weight in childhood, especially in the first two years of life, predict high BP in adolescents. Chiolero et al showed that increased weight gain during any period after birth was associated with high BP in adolescents [13]. Horta et al reported that both early and late catch-up growth were associated with increased systolic BP in adolescents [14]. These studies together with the present study are consistent regarding the influence of rapid weight gain on later BP. The present study further showed that the effect on adolescents' BP was strongest in individuals who were small at birth (weight <2,500g) compared to individuals who weighed ≥2,500g at birth.

Although current weight (a mediator rather than a confounder for the effect of birth weight on later BP [15]) should not be adjusted for when estimating the effect of birth weight on later BP, in this PhD thesis, both results adjusted and not adjusted for the effect of current weight were presented. Results adjusted for current weight enabled easier comparison with results from earlier studies where the effect of birth weight on later BP had been adjusted for. Similar to these earlier studies reviewed in [1, 3, 16], adjusting for current weight in this PhD led to a more negative effect of birth weight on later BP in the

adolescents. In addition to current weight being a mediator, adjusting for current weight makes it complex to interpret the obtained results [3, 15], which could be interpreted as the effect of change in weight between birth and a later age on BP rather that the effect of fetal growth on later BP [15].

Our study suggests that rapid weight gain rather than weight at birth is important in the developmental programming of later BP, with fast-growing low birth weight children at particularly higher risk compared to children who were heavier at birth. This could be due to "a nutritional mismatch" between fetal (the predicted) life and early postnatal (realised) life experienced by individuals who were small at birth and rapidly gain weight in the early post-natal period. In the EMaBS, weight at any time starting at one year of age was a stronger predictor of adolescents' weight than birth weight. This may reflect a resetting of an individual's growth trajectory in the first year of life [15].

8.2.2.3 Blood pressure risk factors in early adolescents

Maternal gestational BMI and educational status and adolescents' age, BMI, family history of hypertension, waist circumference and current infection with *Trichuris* were positively associated with systolic BP. Childhood (<5 years of age) malaria and higher vegetable consumption were associated with reduced systolic BP. Findings for diastolic BP were similar to those for systolic BP, except higher fruit consumption rather than vegetable consumption was associated with lower diastolic BP and there was no association with waist circumference or *Trichuris* infection for diastolic BP. Trial intervention(s) or worm infection(s) in pregnancy or childhood or pre-adolescence had no effect on adolescents' BP.

Maternal factors (smoking in pregnancy, urban residence and socioeconomic status) and adolescent factors (including salt intake, physical activity and sex) associated with high BP in other settings [17-25] were not associated with BP in this study. As in other developing countries, maternal smoking in pregnancy and sedentary lifestyles were uncommon in this population. In this study, nearly all adolescents added salt to cooked food, making it hard to detect a differential effect of salt addition on BP.

Except for maternal education status, other socioeconomic indicators (including socioeconomic index and urbanisation) were not associated with BP. Consistent with earlier studies from Africa and other LICs, the present study has shown that higher maternal education (tertiary or senior) compared to primary was associated with increased BP in adolescents [26, 27]. On the contrary, studies from in HICs have showed

associations between low education status and increased BP [27, 28]. In many developing countries, individuals from more highly educated households are more likely to be obese, live sedentary lifestyles and have unhealthy dietary practices compared to those from less-educated households, whereas the reverse is in HICs.

8.2.2.4 Genetic variants associated with BP

The genetic analysis involved 2,176,694 SNPs from 815 adolescents (with both phenotype and genotypic data). The genetic analysis aimed at identifying genetic variants associated with systolic and, or diastolic BP among Ugandan adolescents aged 10 and 11 years of age. We hypothesised that common genetic variants (unique or not unique to populations in Africa) were associated with systolic and, or, diastolic BP in the adolescents. The BP GWAS revealed no novel variant associated with systolic or diastolic BP. None of the SNPs reached genome-wide significance levels (5×10^{-8}) for systolic or diastolic BP for the GWAS analysis. The most strongly associated variants were: rs29356, p-value = 9.6×10^{-7} for systolic BP and rs73861745, p-value = 9.9×10^{-7} for diastolic BP. For the replication study, 88 SNPs associated with systolic or diastolic BP were identified, of which 74 SNPs had minor allele frequency >0.01; three (4.1%) were replicated with p-value <0.05 but none met a Bonferroni corrected significance threshold of 6.8×10^{-4}.

8.3 Selection into the trial and the Blood Pressure Study

The EMaBS enrolled women who sought antenatal care from Entebbe Hospital. Nearly 70% of pregnant women in the study area seek antenatal care from Entebbe Hospital [29]. Thus, EMaBS did not enroll all of the eligible pregnant women from the study area. A community survey by Millard [30] showed that 60% of mothers in the catchment area would have been eligible for enrollment into the trial, of these 30% had been enrolled into the EMaBS trial. Compared to mothers in the community, those enrolled in the trial were of higher socio-economic and, or educational status.

Twenty seven percent of the pregnant women screened in the EMaBS were eligible for enrollment into the trial, of these, 80% were enrolled. Overall, women enrolled in the trial were similar to those not enrolled for most characteristics, except that those not enrolled were more likely to be primigravida or younger women [31]. Non-enrolment into the EMaBS might have introduced a selection bias, as women at risk of having low birth weight infants were less likely to be enrolled, for example women thought to have an abnormal pregnancy such as those with eclampsia were excluded from the trial. However,

among the children born into the birth cohort, those who participated in the BP study at 10/11 years of age were not different for most characteristics from those who did not participate in the BP study, except that those who did not participate in the BP study were less likely to be HIV positive, but more likely to be singletons and born to more educated or married/cohabiting mothers, who were less likely to have been infected with hookworm at enrolment [32].

The EMaBS children were more likely to be healthy than the children in community: study children were more likely to use footwear or sleep under insecticide-treated bed nets than community children [30], although no difference in incidence rates of reported common infections was observed between children enrolled in the study and those in the community [30]. In addition, EMaBS children received free medical care or their illnesses at the study clinic thus they more likely to have better health seeking behaviours than their community counterparts.

In conclusion, EMaBS participants as a whole, and particularly those included in the BP study were on average, from higher socio-economic or educated backgrounds and likely to be "healthier" than the community they were sampled from. This means that findings may not be fully generalizable to the study setting.

8.4 Interpretation of the study findings

The findings presented in this thesis support earlier observations of no association between intra-uterine growth and adolescent BP [1, 2, 33]; a positive association between increased postnatal growth and elevated BP later in life [4, 34-36]; positive associations between BP and adiposity (both maternal pregnancy and adolescent) [11, 37, 38], family history of hypertension [39] and age [22, 40, 41]; and an inverse association between BP and higher fruit or vegetable consumption [9, 42].

The relationship between accelerated postnatal weight gain and increased adolescent BP, particularly in those small at birth, demonstrates the importance of the 'nutritional mismatch' (slow uterine growth accompanied by accelerated postnatal growth) in the developmental programming of later BP. The strong association in the individuals who were small at birth is suggestive of the effect of IUGR rather than of prematurity on later BP [43, 44].

Findings published in this thesis extend observations to associations between infections and adolescent BP: childhood clinical and or asymptomatic malaria was associated with reduced BP in adolescents while current *Trichuris* infection was associated with increased

BP. Findings of a protective effect of malaria on BP in the present study are consistent with findings in an earlier study among 5- to 18-year-old Ugandans [9]. The protective effect of malaria on later BP is partly mediated through reductions in adolescents' size. In the EMaBS, childhood malaria was associated with later reduction in adolescents' weight or height, findings that are consistent with earlier studies from other malaria endemic areas [45, 46]. Since the 2000s, age-standardized malaria incidence and death rates have declined in most of SSA [47-49], coinciding with the escalating burden of hypertension on the African continent [50]. This could be part of the epidemiological transition on the continent, or the effect could be more direct; the mechanisms remain to be elucidated. However, our findings are contrary to an earlier suggestion from Kenya, of an adverse effect of malaria on BP. Despite seeing an association between sickle-cell trait and malaria in the adolescents in our study, sickle cell trait was not associated with adolescents' BP. Possible explanations for the contrasting findings could be due to differences in study designs and infection intensities. The Kenyan study used sickle cell trait as an instrumental variable for malaria transmission. There are differences in the age distribution of participant and malaria exposure intensity between our study and the Kenyan study. Participants in our study were aged 10 and 11 years and from a malaria endemic area [51], while those in the Kenyan study were over 15 years of age and from Kilifi (currently a low-moderate but historically a high malaria transmission area) and Nairobi (no malaria transmission) [52]. Current infection with *Trichuris* may also contribute to increased BP, but the mechanism is yet to be demonstrated.

A possible explanation for failure of our study to identify genetic variants associated with BP from the GWAS study is that BP is determined by rare variants, each conferring a low to moderate effect on BP. Our study was underpowered to detect associations with rare or low-frequency variants. BP is most likely a polygenic trait influenced by the simultaneous presence of several gene variants each with a small effect size and contributing in an additive manner to BP expression. Thus, the large effect sizes that this study had good power to detect, may not be realistic.

Findings from the genetic study emphasise that BP is most likely due to the simultaneous presence of several gene variants each with a small effect size. The architecture of BP in African adolescents might be different to that in adults, although comparative studies in adults and adolescents would need to be done in order to determine this. Larger studies are required to investigate the genetic variants on BP.

8.5 Study strengths

A strength of this PhD was the availability of well documented prospectively collected data on important covariates (such as birth weight) and potential confounders (such as socioeconomic index) determined before the BP study was conceptualized and designed, minimising potential recall and reporter bias.

Participant retention in this cohort was reasonably good, given the challenges of maintaining such a cohort. The annual loss to follow-up was lower than anticipated at the design stage of the original trial [53]. Overall loss to follow-up was around 50%, less than the 60% level considered to introduce bias if data were missing completely at random or missing at random [54]. However, the data may not satisfy either of these missingness mechanisms. This is a high follow-up for the setting, where address systems are less formal, and mobility is relatively high.

Robust methods were used in defining the main exposure (birth weight), outcomes (BP) and potential confounders (such as BMI, or infections such as HIV). For example, weight scales were regularly calibrated using a standard ten-kilogram stone made for this purpose, while BP monitors were validated and calibrated every six months at the Uganda National Bureau of Standards. BP was measured thrice on a single occasion and on up to two extra occasions in those with BP $\geq 95^{th}$ percentile at the initial visit, to avoid overestimation of high BP as a result of white-coat phenomenon.

Although this was the first-time participants in the EMaBS birth cohort had their BP measured, white-coat phenomenon was probably less of an issue than it would be for participants less familiar with medical and research procedures and settings. Participants in the EMaBS regularly attended this clinic for scheduled and/or illness visits and were familiar with the clinic setting. In an effort to offset effect of white-coat phenomenon, the first BP measurements (higher and different from the second and third readings, see supplementary figure 1, page 125.) were not included the estimation of the mean BP (systolic or diastolic) used for data analysis. Out of office (ambulatory or home-based) BP measurements, would overcome the concerns that arise from white-coat phenomenon and masked hypertension (hypertension not detected during office evaluation but only by out of office evaluation) that arise from office (clinic) BP measurements.

Quality control measures were instituted for various laboratory procedures including malaria microscopy, stool parasitology analyses and HIV testing. Misclassification of these variables was unlikely to be a problem in this cohort.

8.6 Study limitations

The systematic review was based on published studies and hence was prone to publication bias as journals are more likely to publish positive findings than inconclusive or negative findings. However, inconclusive and negative results seem to have also been published, hence if publication bias was an issue, it was not obvious.

The original trial was not designed specifically for this current study on BP, so data on important exposures and potential confounders were missing or not appropriately collected, consequently findings are subject to unmeasured or residual confounding. For example, birth weight was collected but those with low birth weight could not be classified into premature or SGA due to lack of early scan data in this setting. Hence, this study was not in a position to determine the independent effect of prematurity or SGA on later BP. Also, data were missing for some important exposure variables, for example birth weight was missing for 187 (16.7%) of the participants in this current study. Birth weight data was likely not missing at random, as those born away from hospital were more likely to have missing birth weight data, introducing a selection bias. However, EMaBS participants included in the current study were similar in most characteristics to those not included in the study.

Sensitivity analyses assessing the impact of missing values for the main exposures (birth weight and change in WAZ) were conducted, as earlier described in chapter 4. Results from the sensitivity analyses were similar to those from the main analyses. However, simple imputation approaches such as the one used in the thesis are likely to underestimate the standard error, and advanced methods for dealing with missing data (such as multiple imputation) would have improved these sections of the thesis [55]. An imputation approach was used for genetic data but not for the analyses of environmental and life-style factors presented in chapters 5 and 6. This was a limitation of the thesis.

Pregnant women with evidence of helminth-induced pathology or history of adverse reaction to anthelminthics or abnormal pregnancy were excluded at enrolment. These may have introduced a selection bias in the study population, as those at increased risk of having low birth weight neonates were most likely not enrolled in the original trial.

There are no standardised BP thresholds for young Africans, thus this PhD used the American BP thresholds for children and adolescents to estimate the prevalence of pre-hypertension and hypertension. This permitted comparison of pre-hypertension or hypertension prevalence across different studies and populations. A fundamental

limitation to the use of these thresholds is, they were defined using and for the American population which is different in many ways, with higher average WAZ, WHZ and HAZ compared to African populations [56], such as those participants included in the current BP study.

The multivariable analysis in chapter 6 might have been underpowered to detect effects of some of the exposures due to the large number of risk factors included in some models. This may especially have been the case for the proximal factors (these were added last to the model, hence analyses for these factors were based on models with a particularly large number of predictor variables, which could have led to overfitting and consequently reduced power). In addition, a large number of statistical tests were undertaken; thus, some findings may be due to multiplicity. However, it is reassuring that most findings are consistent with previous literature, albeit from different settings.

The use of microscopy for malaria diagnosis might have resulted in differential misclassification of malaria. In malaria endemic areas, asymptomatic malaria often presents as sub-microscopic in individuals with past malaria infection [57]. Sub-microscopic malaria was most likely missed and classified as no malaria in individuals with past malaria infection(s), thus underreporting the proportion with asymptomatic malaria at a given annual study visit.

Some important exposures and potential confounders (including diet, salt intake, physical activity) were self-reported, thus subject to recall bias.

8.7 Generalisability of findings

Findings in the present study may apply to the wider Ugandan population. Overall, no major differences existed between cohort members enrolled and not enrolled in the BP study. The findings may be generalisable to a wider African population where malaria is endemic, with moderate prevalence of low birth weight and low prevalence of hypertension.

However, findings in the current study may not apply to other populations. Participants in this cohort received free medical care so may have had better health outcomes than the general population. In addition, our findings may not be applicable to other settings with dissimilar exposure or outcome frequencies such as the prevalence of hypertension, low birth weight, worm infections and malaria. For example, Entebbe is a malaria endemic region with moderate to high malaria transmission [51, 58], and in the present study prevalence of low birth weight and hypertension was low at 7% and 1%, respectively.

Findings in this thesis may not apply to non-malaria endemic areas with higher low birth weight prevalence or higher prevalence of hypertension compared to the EMaBS study area.

Adolescents with normal or pre-hypertension on the first occasion were not invited for extra BP measures, this might have resulted in an under estimation of the overall hypertension prevalence in our study. The overall prevalence of hypertension might have been higher if all participants had their BP measured on a second and third occasion, regardless of the value on the first occasion.

8.8 Implications for research

The influence of intra-uterine growth on later BP among young Africans remains understudied. There is the need for more studies (with appropriate sample size and applying appropriate data analysis methods controlling for potential confounders) from Africa investigating the influence of both intra-uterine growth and postnatal growth on later BP.

Findings reported in this thesis should be replicated using further cohort studies or alternative study designs. The impact of birth weight, growth, infections (including malaria and *Trichuris*) and genetic variants on BP could be investigated using designs such as individual participant data (IPD) meta-analysis or Mendelian randomization (MR). Several small African cohorts could contribute data for IPD meta-analyses to enable investigation of the suggested U-or J-shaped relationship between birth weight and later BP; effect of infections on BP and also improve power for conducting subgroup analyses, for example, for variables such as season of conception or birth and prematurity or IUGR.

The MR design can be used to understand the relationship between exposures (such as birth weight, malaria) and later BP. MR studies use genetic variant(s) with known biological effects as instrumental variables (to represent a known environmental exposure) to confirm or refute associations between the environmental exposure and BP. MR assumes there are no confounders for the association between the selected instrumental variable and the outcome and no pleiotropy (a gene influencing two or more unrelated phenotypic traits). The MR design would enable inference where randomised trials cannot be conducted due to lack of feasibility or ethical equipoise.

Low birth weight is either due to prematurity or IUGR, both of which have different determinants [59, 60]. Thus, combining prematurity and IUGR together as low birth

weight might hinder development of preventive interventions. Birth weight is easily measured with good validity and precision; thus, researchers continue to use low birth weight instead of prematurity or IUGR in studying their impact on later disease. Assessment of prematurity or IUGR involves accurate estimation of gestational age, which is often difficult in developing countries. In developing countries (including Uganda), IUGR constitutes most of the low birth weight in neonates [61]. In the present study, it is not clear if prematurity or IUGR is associated with BP later in life because data on prematurity and IUGR was not collected. Future work on the relationship between intra-uterine growth and BP should aim to assess the independent effect of prematurity or IUGR on later BP. Understanding the possible underlying mechanism through which birth weight impacts later BP requires knowing if prematurity or IUGR have similar or different associations with BP later in life.

In Africa where malnutrition is prevalent, the impact of catch-up nutrition in malnourished infants or infants born small on BP later in life is understudied. Future research should seek to evaluate the impact of catch-up nutritional programmes in malnourished infants or infants born small on the programming of later BP.

Future work should take advantage of various small and large cohorts from Africa to form consortia that can enable the conduct of GWAS meta-analysis well powered to identify rare and low-frequency variants that may be associated with BP. Polygenic scores capture a greater proportion of explained variance in the target phenotype than a single variant. The use of polygenic scores could help explain the missing BP heritability. However, this requires identification of independent variants from well powered GWAS and use of SNPs common to both the discovery and target samples. Future candidate gene analysis using a sample from a different geographical region or ethnic background should investigate for interactions between variants, this might help our understanding of the aetiology of BP. It is possible that multiple interacting genetic variants (rare and common) are influencing BP levels in this population.

8.9 Policy implications and challenges

Findings in this PhD highlight the importance of optimal growth, weight reduction and a healthy diet in high BP prevention. A BP control and high BP prevention policy or strategy should adopt a life-course approach, starting in pre-conception or early pregnancy, through childhood and adolescence into adulthood. Such a BP control and prevention policy would require implementation of multi intervention evidence-based

interventions, aiming at increasing physical activity, reducing obesity and promoting a healthy diet. A school health programme promoting physical exercise and a healthy diet, in addition to educating the next generation of Ugandans might provide a good platform for behavioural change aimed at controlling and preventing high BP. However, behavioural intervention programmes have historically struggled to create long-lasting changes in lifestyle.

Physical exercise in pregnancy is associated with health benefits both for the mother (lower risk for gestational obesity or hypertensive disorders of pregnancy) and their resulting offspring (normal birth weight infants) [62, 63]. Despite evidence that exercise in pregnancy does not increase the risk of preterm births or reduce the mean gestational age at birth [63], implementing a policy advocating for exercise in pregnancy is likely to face fears of anticipated pregnancy loss following physical exercise.

Commercialisation of physical exercise through gym subscriptions, school clubs and sports academies, in addition to decreasing public play grounds and facilities is a major challenge to implementing a physical exercise policy. In addition, schools are paying greater attention to final year results than the physical wellbeing (keeping active) of their learners. Physical education lessons are no longer part of the curriculum in most schools in Uganda and sport facilities are replaced with class room facilities. Despite recommendations by the Uganda ministry of education and sport that schools should have play grounds, many schools do not have play grounds or facilities.

Our study has showed that enhanced weight gain in those small at birth was associated with higher BP later in life. However, in a country with a high prevalence of undernourished infants, a policy preventing enhanced weight gain may be controversial. The short-term impact on mortality from malnutrition (kwashiorkor or marasmus) outweighs the longer-term impact of rapid weight gain on increased risk for later higher BP. Although a policy change advocating for normal rather than rapid weight gain (catch-up) growth may have a long-term impact on later BP it would require development of clear definitions for slow, normal and rapid weight both for the malnourished children and those born with a low birth weight, and even then, may not be considered acceptable by parents and clinicians.

Despite international recommendations for at least one BP measurement every year in heathy individuals and at every hospital/clinic visit for those at increased risk of BP, most clinicians do not measure BP in children and adolescents. A policy change is needed that includes training of medical students to actively screen for BP and accurately make a

diagnosis of high BP in children and adolescents. However, if clinicians are to screen and accurately diagnose high BP, appropriated sized BP cuffs and simplified BP tables should be available, accessible and used.

The inverse association between fruit or vegetable consumption and BP observed in this study strengthens the need for educating children, families and schools on regular consumption of fruits and vegetables. A food and diet policy targeting both commercial and processing industries should aim to promote heathy dietary practices through adequate consumption of fruits and vegetables; reduction in daily salt intake to less than 6 grams (which could prevent 2.5 million deaths globally per year); reduction of sugar and salt in processed food and beverages and adequate labelling of food content for processed foods. The growth in fast- and street-food, which are low cost, easily accessible and richer in salt and fat (saturated and trans-fatty acid) than traditional foods presents a great challenge in promoting a healthy diet in Uganda.

Socio-economic and cultural beliefs regarding being overweight or obese are a challenge to the implementation of BP control and prevention policy. In many African societies, parents consider obese children as healthily children with no concerns or problems. Being of a large size is a sign of beauty, "good feeding", prosperity, good health and wellbeing, and prestige, whereas small body size is sometimes considered a sign of poverty and poor health [64].

8.10 Conclusion

The systematic review showed that the relatively small number of studies with different designs prohibited definitive conclusions. The relationship between birth weight and BP among young African remains understudied and appears to vary depending on the age of the participant. The observational study has demonstrated that i) both low and high birth weight might be associated with later BP, ii) rapid early postnatal weight gain especially in small individuals at birth plays a key role in the developmental programming of later BP; and iii) malaria eradication might escalate the burden of high BP. Multiple interventions initiated early in life targeting individuals with family history of hypertension, aiming at reducing adiposity (in pregnancy and adolescence) and promoting fruit and vegetable consumption could be essential in the control of adulthood hypertension and subsequent CVDs. It remains unclear how much of the variation in BP among adolescents in SSA can be attributed to genetic factors.

8.11 References

1. Lule, S.A., et al., Is birth weight associated with blood pressure among African children and adolescents? A systematic review. J Dev Orig Health Dis, 2018. **9**(3): p. 270-280.
2. Law, C.M. and A.W. Shiell, Is blood pressure inversely related to birth weight? The strength of evidence from a systematic review of the literature. J Hypertens, 1996. **14**(8): p. 935-41.
3. Hardy, R., et al., Birthweight and blood pressure in five European birth cohort studies: an investigation of confounding factors. Eur J Public Health, 2006. **16**(1): p. 21-30.
4. Huxley, R.R., A.W. Shiell, and C.M. Law, The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. J Hypertens, 2000. **18**(7): p. 815-31.
5. Barker, D.J., et al., Fetal and placental size and risk of hypertension in adult life. BMJ, 1990. **301**(6746): p. 259-62.
6. Shankar, R.R., et al., The change in blood pressure during pubertal growth. J Clin Endocrinol Metab, 2005. **90**(1): p. 163-7.
7. Tu, W., et al., Synchronization of adolescent blood pressure and pubertal somatic growth. J Clin Endocrinol Metab, 2009. **94**(12): p. 5019-22.
8. Monyeki, K., et al., Association between Blood Pressure and Birth Weight among Rural South African Children: Ellisras Longitudinal Study. Int J Environ Res Public Health, 2017. **14**(9).
9. Kidy, F., et al., Blood pressure in primary school children in Uganda: a cross-sectional survey. BMC Public Health, 2014. **14**: p. 1223.
10. Chiolero, A., et al., Prevalence of elevated blood pressure and association with overweight in children of a rapidly developing country. J Hum Hypertens, 2007. **21**(2): p. 120-7.
11. Jobe, M., et al., High blood pressure and associated risk factors as indicator of preclinical hypertension in rural West Africa: A focus on children and adolescents in The Gambia. Medicine (Baltimore), 2017. **96**(13): p. e6170.

12. Oyewole, O.O. and K.S. Oritogun, Pre-hypertension and hypertension in adolescence: how much does it occur in a Nigerian community? West Afr J Med, 2012. **31**(2): p. 71-5.

13. Chiolero, A., et al., Birth weight, weight change, and blood pressure during childhood and adolescence: a school-based multiple cohort study. Journal of Hypertension, 2011. **29**(10): p. 1871-1879.

14. Horta, B.L., et al., Early and late growth and blood pressure in adolescence. J Epidemiol Community Health, 2003. **57**(3): p. 226-30.

15. Blake, K.V., et al., Adjustment for current weight and the relationship between birth weight and blood pressure in childhood. J Hypertens, 2000. **18**(8): p. 1007-12.

16. Huxley, R., A. Neil, and R. Collins, Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? Lancet, 2002. **360**(9334): p. 659-65.

17. Beratis, N.G., D. Panagoulias, and A. Varvarigou, Increased blood pressure in neonates and infants whose mothers smoked during pregnancy. J Pediatr, 1996. **128**(6): p. 806-12.

18. Afrifa-Anane, E., et al., The association of physical activity, body mass index and the blood pressure levels among urban poor youth in Accra, Ghana. BMC Public Health, 2015. **15**: p. 269.

19. Chen, J.L., et al., Risk factors for obesity and high blood pressure in Chinese American children: maternal acculturation and children's food choices. J Immigr Minor Health, 2011. **13**(2): p. 268-75.

20. Creating an enabling environment for population-based salt reduction strategies: report of a joint technical meeting held by WHO and the Food Standards Agency, United Kingdom, Geneva, World health Organization, 2010.

21. Ansa, V.O., et al., Relationship between parental socio-economic status and casual blood pressure in coastal Nigerian adolescents. West Afr J Med, 2010. **29**(3): p. 146-52.

22. Addo, J., L. Smeeth, and D.A. Leon, Hypertension in sub-saharan Africa: a systematic review. Hypertension, 2007. **50**(6): p. 1012-8.

23. Guwatudde, D., et al., The Epidemiology of Hypertension in Uganda: Findings from the National Non-Communicable Diseases Risk Factor Survey. PLoS One, 2015. **10**(9): p. e0138991.

24. Nkeh-Chungag, B.N., et al., Prevalence of hypertension and pre-hypertension in 13-17 year old adolescents living in Mthatha - South Africa: a cross-sectional study. Cent Eur J Public Health, 2015. **23**(1): p. 59-64.

25. Paciencia, I., et al., Association between sleep duration and blood pressure in adolescents. Hypertens Res, 2013. **36**(8): p. 747-52.

26. Caballero, B., The global epidemic of obesity: an overview. Epidemiol Rev, 2007. **29**: p. 1-5.

27. Chow, C.K., et al., Prevalence, awareness, treatment, and control of hypertension in rural and urban communities in high-, middle-, and low-income countries. JAMA, 2013. **310**(9): p. 959-68.

28. Leng, B., et al., Socioeconomic status and hypertension: a meta-analysis. J Hypertens, 2015. **33**(2): p. 221-9.

29. Webb, E.L., et al., Effect of single-dose anthelmintic treatment during pregnancy on an infant's response to immunisation and on susceptibility to infectious diseases in infancy: a randomised, double-blind, placebo-controlled trial. Lancet, 2011. **377**(9759): p. 52-62.

30. Millard, J.D., et al., Assessing the external validity of a randomized controlled trial of anthelminthics in mothers and their children in Entebbe, Uganda. Trials, 2014. **15**: p. 310.

31. Woodburn, P.W., et al., Risk factors for helminth, malaria, and HIV infection in pregnancy in Entebbe, Uganda. PLoS Negl Trop Dis, 2009. **3**(6): p. e473.

32. Lule, S.A., et al., Are birthweight and postnatal weight gain in childhood associated with blood pressure in early adolescence? Results from a Ugandan birth cohort. Int J Epidemiol, 2018.

33. Woelk, G.B., Is low birth weight a risk factor for adult hypertension? A literature review with particular reference to Africa. South African Medical Journal. Suid-Afrikaanse Tydskrif Vir Geneeskunde, 1995. **85**(12 Pt 2): p. 1348-9, 1352-3.

34. Cournil, A., et al., Enhanced post-natal growth is associated with elevated blood pressure in young Senegalese adults. Int J Epidemiol, 2009. **38**(5): p. 1401-10.

35. Belfort, M.B., et al., Size at birth, infant growth, and blood pressure at three years of age. J Pediatr, 2007. **151**(6): p. 670-4.

36. Bowers, K., et al., Birth weight, postnatal weight change, and risk for high blood pressure among chinese children. Pediatrics, 2011. **127**(5): p. e1272-9.

37. Sorof, J. and S. Daniels, Obesity hypertension in children: a problem of epidemic proportions. Hypertension, 2002. **40**(4): p. 441-7.

38. Willig, A.L., et al., Adjusting adiposity and body weight measurements for height alters the relationship with blood pressure in children. Am J Hypertens, 2010. **23**(8): p. 904-10.

39. Okoh, B.A.N. and E.A.D. Alikor, Childhood hypertension and family history of hypertension in primary school children in Port Harcourt. Niger J Paed, 2013. **40**(2): p. 184-188.

40. Zinner, S.H., et al., Significance of blood pressure in infancy. Familial aggregation and predictive effect on later blood pressure. Hypertension, 1985. **7**(3 Pt 1): p. 411-6.

41. Bovet, P., et al., Prevalence of cardiovascular risk factors in a middle-income country and estimated cost of a treatment strategy. BMC Public Health, 2006. **6**: p. 9.

42. Pienovi, L., et al., [Fruit and vegetable intake, and blood pressure. A population research]. Arch Latinoam Nutr, 2015. **65**(1): p. 21-6.

43. Juonala, M., et al., Effect of birth weight on life-course blood pressure levels among children born premature: the Cardiovascular Risk in Young Finns Study. J Hypertens, 2015. **33**(8): p. 1542-8.

44. Demicheva, E. and F. Crispi, Long-term follow-up of intrauterine growth restriction: cardiovascular disorders. Fetal Diagn Ther, 2014. **36**(2): p. 143-53.

45. ter Kuile, F.O., et al., Impact of permethrin-treated bed nets on malaria, anemia, and growth in infants in an area of intense perennial malaria transmission in western Kenya. Am J Trop Med Hyg, 2003. **68**(4 Suppl): p. 68-77.

46. Bradley-Moore, A.M., et al., Malaria chemoprophylaxis with chloroquine in young Nigerian children. III. Its effect on nutrition. Ann Trop Med Parasitol, 1985. **79**(6): p. 575-84.

47. Bhatt, S., et al., The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature, 2015. **526**(7572): p. 207-211.

48. Noor, A.M., et al., The changing risk of Plasmodium falciparum malaria infection in Africa: 2000-10: a spatial and temporal analysis of transmission intensity. Lancet, 2014. **383**(9930): p. 1739-47.

49. Murray, C.J., et al., Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet, 2014. **384**(9947): p. 1005-70.

50. Dalal, S., et al., Non-communicable diseases in sub-Saharan Africa: what we know now. Int J Epidemiol, 2011. **40**(4): p. 885-901.

51. Odongo-Aginya, E., et al., Relationship between malaria infection intensity and rainfall pattern in Entebbe peninsula, Uganda. Afr Health Sci, 2005. **5**(3): p. 238-45.

52. Etyang, A.O., Determining the Causal Role of Malaria in Elevating Blood Pressure and Pulse Wave Velocity in Kenyan Adolescents and Adults, in Department of Infectious Disease Epidemiology. 2017, London School of Hygiene & Tropical Medicine.

53. Elliott, A.M., et al., The impact of helminths on the response to immunization and on the incidence of infection and disease in childhood in Uganda: design of a randomized, double-blind, placebo-controlled, factorial trial of deworming interventions delivered in pregnancy and early childhood [ISRCTN32849447]. Clin Trials, 2007. **4**(1): p. 42-57.

54. Kristman, V., M. Manno, and P. Cote, Loss to follow-up in cohort studies: how much is too much? Eur J Epidemiol, 2004. **19**(8): p. 751-60.

55. Sterne, J.A., et al., Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ, 2009. **338**: p. b2393.

56. Corsi, D.J., J.M. Perkins, and S.V. Subramanian, Child anthropometry data quality from Demographic and Health Surveys, Multiple Indicator Cluster Surveys, and National Nutrition Surveys in the West Central Africa region: are we comparing apples and oranges? Glob Health Action, 2017. **10**(1): p. 1328185.

57. Lin, J.T., D.L. Saunders, and S.R. Meshnick, The role of submicroscopic parasitemia in malaria transmission: what is the evidence? Trends Parasitol, 2014. **30**(4): p. 183-90.

58. Yeka, A., et al., Malaria in Uganda: challenges to control on the long road to elimination: I. Epidemiology and current control efforts. Acta Trop, 2012. **121**(3): p. 184-95.

59. Delnord, M., B. Blondel, and J. Zeitlin, What contributes to disparities in the preterm birth rate in European countries? Curr Opin Obstet Gynecol, 2015. **27**(2): p. 133-42.

60. Shulman, C.E. and E.K. Dorman, Importance and prevention of malaria in pregnancy. Trans R Soc Trop Med Hyg, 2003. **97**(1): p. 30-5.

61. Garner, P., M.S. Kramer, and I. Chalmers, Might efforts to increase birthweight in undernourished women do more harm than good? Lancet, 1992. **340**(8826): p. 1021-3.

62. Jiang, H., et al., Can physical activity reduce excessive gestational weight gain? Findings from a Chinese urban pregnant women cohort study. Int J Behav Nutr Phys Act, 2012. **9**: p. 12.

63. Wang, C., et al., A randomized clinical trial of exercise during pregnancy to prevent gestational diabetes mellitus and improve pregnancy outcome in overweight and obese pregnant women. Am J Obstet Gynecol, 2017. **216**(4): p. 340-351.

64. Mansur, A.J., et al., [Infective endocarditis: analysis of 300 episodes]. Rev Port Cardiol, 1992. **11**(12): p. 1067-76.

Appendix 1: LSHTM ethical notification

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

www.lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Observational / Interventions Research Ethics Committee

Dr Abubaker Swaib Lule
LSHTM

9 June 2016

Dear Abubaker

Study Title: Blood pressure and cardiovascular disease risk factors among 10 and 11 year old children in the Entebbe Mother and Baby Study

LSHTM Ethics Ref: 11253

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Covering Letter	UVRI Science and Ethics committee approval_27Nov2013	26/11/2013	1.0
Covering Letter	UNCST_letter	10/01/2014	1.0
Local Approval	1. Letter	19/02/2014	1.0
Protocol / Proposal	07_MAB_HAEMATOLOGY _form	21/03/2014	1.0
Protocol / Proposal	07_MAB_ BIOCHEMISTRY FORM	08/05/2014	1.0
Protocol / Proposal	07_MAB_urine form	08/05/2014	2.0
Covering Letter	02_assent_english	27/06/2014	2.0
Covering Letter	02_assent_luganda	27/06/2014	2.0
Covering Letter	02_consent_English	27/06/2014	2.0
Covering Letter	02_consent_Luganda	27/06/2014	2.0
Local Approval	2. application	04/09/2014	1.0
Protocol / Proposal	03_BPS_CRF	06/03/2015	2.0
Protocol / Proposal	Blood pressure study	16/06/2015	1.1
Covering Letter	UVRI REC_2.0	02/07/2015	2.0
Protocol / Proposal	03_BPS_BCA	29/07/2015	1.0
Investigator CV	1.2015_CV	23/03/2016	1.1
Covering Letter	Letter	26/05/2016	1.0

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

Appendix 2: Information sheet and assent form (English version)

Version 2.0 27th June 2014

Uganda Virus research Institute/Entebbe Hospital

Information sheet for children

Blood pressure study among 10 and 11 year old children in the Entebbe Mother and baby study

Introduction

Thank you for taking part in the earlier studies in the Entebbe Mother and Baby study (EMaBS). This has helped us collect a lot of information about health of children and their mothers. At this visit we would like to measure your blood pressure. This will help us to find out what factors early in life can affect a person's blood pressure.

What is blood pressure?

Blood pressure is the pressure of the blood inside your blood vessels. The more blood your heart pumps and the narrower your blood vessels, the higher your blood pressure. A person can have high blood pressure for a long time without noticing any problem, but high blood pressure can cause a lot of health problems in the long run if it is not treated.

Why is this study being done?

High blood pressureis fast growing problem in developing countries and not much is known about the reasons for this. EMaBS children can help us to find out.

Who is doing the study?

The EMaBS togetherwith Entebbe Hospital are carrying out this study and it will end in 2016.

Why have you chosen me?

We have chosen you because you are 10 or 11 years of age and under follow up the EMaBS. We have collected a lot of information about your health from the time you were in your mother's womb to date. We think that this information, together with information about your blood pressure, will help usto answer questions about blood pressure in children from developing countries.

What will happen if I take part?

Some EMaBS activities will continue, just as before:

- You will be seen,ask questions about your health, and measure your height and weight
- We will also ask you to provide a stool sample.
- After you have given a stool sample we will give you treatment for worms. If the results show that you need special treatment, one of the fieldworkers will come and bring the treatment to your home.

If you agree to take part in this study about blood pressure when you are 10 or 11 years of age, this is what willhappen.

- As well as height and weight, we will measure the size of your waist. We will then take your blood pressure and this will be done three times on the first day. Quite often the reading is high on the first day. This should not frighten you. It can happen just because you are not used to having your blood pressure measured. If your blood pressure is high on the first day we will invite you to come back so that it can be measured again on a second and third day.
- The results of you blood pressure will be compared to the normal values for your age, sex and height.
- A blood sample (14mls, about three smallteaspoons) will be taken.
- A urine sample will be collected and stored.
- We will combine all of this information with other information we have collected from you since your birth including information about your genes. We will look for genes that may affect your blood pressure and heart and blood vessels
- You will be refunded your transport cost.
- If your blood pressure is found to be too high, some extra tests will be done to find out why this is and we will arrange for you to see a specialist doctor who can help you..

What will the stool and blood samples be used for?

The stool and blood samples will be tested for infections. The blood sample will also be tested for chemical changes such as sugar and lipid (fat) levels: high sugar levels and abnormal fat levels quite often occur alongsideproblems with blood pressure. Some of the blood sample, as well as the urine sample, will be stored for other tests in the future. These may include measurement of substances involved in the immune system and nutrition. All the information collected will be linked to your information that we already have, including the information about your genes. This information and results of the test will all be kept safely and will be used only for research and to help us to look after your health. The results of the sugar and fat levels will be available to you and if they are not normal you will be advised what to do.

Will this cause any problem to me?

Taking to part in this study is not expected to cause any problems to you. Measuring blood pressure and taking a blood sample may be a little bit uncomfortable to you. But this is temporary and not expected not to have any danger to you.

Are there any benefits to taking part in the study?

It is good to know your blood pressure. Most doctors check blood pressure for their patients as a routine. Results of the blood tests will also be useful for your health.

There is a very small possibility that we may discover some genes that increase the risk of you having high blood pressure or related diseases which may affect your health as you get older or the health of

any children you may have. If we discover that you have such genes we will make every effort to contact you or your parents and provide appropriate guidance with what to do. We think the chances of this happening are very small

It is your right to refuse or withdraw from the study.

Taking part in this study is voluntary. This means that you or the person who is looking after you can say that you do not want to be involved. You can change your mind about taking part in the study at any time.

You can find out more about this study at any time by asking one of the study doctors, nurses or field workers at the clinic. You can also contact Dr. Elliott (telephone: 0417704000) or the Medical Superintendent of Entebbe Hospital (telephone: 320058), or the Ethics Committee Chairman from Uganda Virus Research Institute on 0414 321962.

Version 2.0 27th June 2014

Uganda Virus research Institute/Entebbe Hospital

Child Assent form

Study of blood pressure among school aged children in the Entebbe Mother and baby study (EMaBS)

Child's name..... ..... Child's BIDNO |___|___|___|___|/|___|

My signature below shows that I have read and/or been fully explained the information sheet concerning my participation in this study and I understand what will be required if I take part in the study.My questions concerning this study have been answered. My participation is voluntary and I agree to participate in this study.

Child's signatures:

1. My signature/thumb print indicates that I agree to take part in the blood pressure study

Name (Block Capitals) Signature or thumb print Date

.....

2. My signature/thumb print indicates that I agree for part of my specimens to be stored for future studies

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....

Witness;

....

Witness required only for those using a thumb print, or unable to read the information and assent form, or if the person taking the assent does not speak the participant's language. The witness must not be a member of the research team or a study participant but may be a family member or a member of hospital staff who is not involved in the research programme. The witness much be present for the whole consent process.

Person conducting the assent:

....

Name (Block Capitals) Signature Date

Appendix 3: Information sheet and assent form (Luganda version)

Version 2.0 27th June 2014

Uganda Virus research Institute/Entebbe Hospital

Olupaulalw'obubakabw'abaana

Okunnonyerezakw'entununsi mu baanaeb'emyakaekkumi (10) n'ekkumin'ogumu (11) mukibiinakyabamaaman'abaanaekya Entebbe Mother and Baby Study (EMaBS).

Ennyanjula

Webalekwenyigira mu kunonyerezaokwasookaokw'ekibiinakyabamaaman'abaana (EMaBS). Kino kituyambyeokukunganyaamawuliremangiagakwatakubulamubw'abaana ne bamaamababwe. Ku lukyalalunotwandyagaddeokupimaentununsizo.Kino kijjakutuyambaokuzuulabiki mu bulamubw'omuntung'akyalimutoebiyinzaokutataganya (okukosa) entununsize.

Entununsikyeki?

Entunnunsig'emanyig'omusaayikweguddukira mu misuwagyo.Omutimagwogyegukomyaokusundaomusaayi ate n'emisuwagyogyejikomyaobufunda, n'entunnunsizogyezikomyaokweyongerawaggulu.
Omuntuasobolaokubeeran'etunnunsieziriwaguluokumalaekiseerakiwanvungatalinakizibukyalabye, nayeentununsizisobolaokuleetaebizibuby'obulamubingi mu maasoeyobwezibatezijjanjabiddwa.

Lwakiokunonnyerezakunokukolebwa?

Ekzibuky'entunnunsikyeyongeddennyo munsiezikyakula ate ngatewaliky'amanyikimanyiddwaekileetakino. AbaanabaEMaBSbayinzaokutuyambakookuzuulakino

Aniakolaokunonyerezakuno?

Aba EMaBSwamun'ab'eddwaliro Entebbe be kola okunonyerezakuno era kujjakuggwa mu 2016.

Lwakimulonzenze?

TukulonzekubangaolinaemyakaKkumi (10) obakkuminagumu (11) atengaoli mu kibiinakyaEMaBS. Tukunganyizzaamawuliremangikubulamubwo mu kiseeeralwewali mu lubuutolwamaamawookutuusakati. Tulowoozantiamawuliregano, n'agoag'entunnunsizo, gajjakutuyambaokwanukulaekibuuzokubulwaddebw'entunnunsi mu baana mu mawangaagakyakula

Kiki ekinabawosinganetabamu?

Ebimukubikolebwa mu EMaBSbijjakweyongera, era ngabulijjo:

- Ojjakulabibwa, obuuzeebibuuzokubulamubwo, era opimibweobuwanvun'obuzito
- Tujja era kukusabaoleeteekyolooni.

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1

- Bwonomalaokuleetaekyoloonitujjakukuwaeddagalaly'ebiwukaby'omulubuto. Ebiva mu kukeberabwebiragantiwetaagaobujjanjabiobw'enjawulo, omukubasawobaffeajjakukuleetaobujjanjabiawakawamwe.

Bwonobaokkirizaokwetaba mu kunononyerzakw'entununsing'oliwamyakakkumi(10) obakkuminagumu (11), bino bye bijjaokubaawo.

- Ngakubuwanvun'obuzito, tujjakupimaekiwatokyo. Tujja era kupimaentunnunsizo, era kinokijjakukolebwaemirundiesatu (3)kulunakuolusooka. Emirundiegisingaebisomebwakuntunnunsibiragantibiliwaggulukulunakuolusooka. Kino tekirinakukutiisa. Kisobolaokubeerawakubangatomanyiddekukupimantunnunsizo. Singaentunnunsizoziriwaggulukulunakuolusookatujjakukuyitaokomewotusoboleokukupimanate kulunakuolw'okubiri(2) era n'olw'okusatu (3).
- Ebiva mu kukupimaentunnunsibijjakugerageranyizibwakw'ebyoebitekwaokubeeraebituufueby'emyakagyo , ekikulakyo era n'obuwanvubwo.
- Tujjakukujjakosampoloy'omusaayiobujiikobwakyayiobutunongabusattu(3) (14mls).
- Sampoloy'omusuloejjakukujjibwakoeterekebwe.
- Tujjakugattaamawulireganogonnawamua'agogetukukungayizzakookuvangawakazalibwangakwo tadden'ebyoebikwatakubuzalilannwabwoobw'endagabutonde. Tujjakunonyaendagabutondoeziyinzaokutataganyaentunnunsizon'omutima era n'emisuwa.
- Ojjakuddizibwawosentez'entabula.
- Singakizulibwantientunnunsizoziriwaggulunnyo, tujjakukolayookukeberaokulalaokuzuulalwakikiribwekityo era tujjakukolaentekatekaolabeomusawoomukuguayinzaokukuyamba.

Sampoloz'omusaayin'ekyolonizakukozesaki?

Sampoloz'omusaayin'ekyolonizijjakweyambisibwaokukeberaendwaddeendala.Sampoloy'omusaayiejjak weyambisibwaokukeberaendwaddeenddalagambangassukaalin'amasavu; ssukaaling'aliwagulun'amasavuamangibiteraokubeerawokumun'ebizibuby'entunnunsi. Sampoloz'omusaayiezimu era ne sampoloy'omusulozijjakuterekebwazeyambisibwe mu kukeberaokwenjawulo mu biseeraeby'omumaaso.Binobiyinzaokutwakimun'okukeberaebintuebirarangaamanyig'omubirin'ebyendii sa.Amawuliregonnagetunafunagajjakuyungibwaku ago getwakukunganyakoeddagetulinangamwemulin'agoagakwatakundagabutondezo.

Amawulireganon'ebividde mu kukeberebwabyonnabijjakweyabisibwa mu kunonyerezakwoka era n'okutunyambaokulabiriraobulmubwo.Ebiva mu kukeberassukaalin'obungibw'amasavubijjakukuwebwa era singatebibabilungi, ojjakuwebwaamagezikukikieky'okukola.

Kino kinandeteraobuzibubwonna?

Okwetaba mu kunonyerezakunotekisuubirwakukuleeterabuzibubwonna.Okupimaentunnunsin'okukujjakosampoloz'omusaayikiyinzaokukukalubirizamu.Nayekinokyakaseerakatono era tekisuubirwakukuleteramutawanagwonna.

Waliwoemiganyulogyonna mu kwetabamukunonyerezakuno?

Kirungiokumayaentunnunsizo.Abasawoabasingabapimaentunnunsiz'abalwaddebabweng'enkolaeyabulijjo.Ebiva mu kukeberakw'omusaayi era n'abyobijjakubabyamugasoeriobulamubwo.

Waliwookuteberezaokutonotononontituyinzaokuzuulaendagabutondeezongezakumikisagy'entunnunsiekiyinzaokukosaobulamubwobulilw'ogendaokulaobaobulamubw'omwanayennagw'olizaala.Singatukizuulantiolinaendagabutondeng'ezo, tujjakukolakyonnaekisobokaokukutuukiriraobaabazaddebotusoboleokukuwaamagezikukikieky'okukolaekituufu.Tulowoozantiemikisagyakinookubeerawomitonoddala

Ddembelyookugaanaobaokukkirizaobaokuva mu kunonyerezakuno.

Okwetabakwo mu kunonyerezakunokwakyeyagalire.Kinokitegeezantiggweobaomuntuakulabiriraasobolaokugambantitoyaglakwetabamu.Oyinzaokukyusa mu ndowoozakuky'okwetabamukunonyerezaekiseerakyonna.

Oyinzaokumanyaebisingawokukunonyerezakunoekiseerakyonnang'obuuzaomukubasawob'okunonyereza, banansiobaabakozikukilinika.Oyinza era okutukuriraDokita Elliott (essimu:0417704000) obaomukuluw'eddwaliroly'Entebbe (Essimu 0414320058), obaomukuluw'akakiiko aka Sayansin'empissaokuva mu Uganda Virus Research Institute kussimu 0414321961.

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Uganda Virus research Institute/Entebbe Hospital

Okukkirizakw'omwana

Okunonyerezakubulwaddeb'entunnunsi mu baanaab'emyakaegigenda mu ssomero mu kibiinakyabamaaman'abaana (EMaBS)

Erinnyaly'omwana.....Ennambay'omwanaBIDNO |___|___|___|___|___|/|___|

Omukonogwangewamangagulaga anti nsomyeobannyinnyonyoddwabulungiolupapulalw'obubakaobukwatakukwetabakwange mu kunonyerezakuno era ntegeerabikiebijjaokwetagisasinganetaba mu kunonyerezakuno.Ebibuuzobyangeebikwatakukukunonyerezakunobiddiddwamu.Okwetabamukwangekwakyeyagalire era nzikirizaokwetaba mu kunonyerezakuno.

Emikonogy'omwana:

1. Omukonogwange/ekinkumukiragantinzikirizaokwetabamukunonyerezakw'obulwaddebw'entunnunsi

Erinnya (mu nukutaennene) **Omukonoobaekinkumuennakuz'omwezi**

....

2. Omukonogwange/ekinkumukiragantinzikrizaebimukw'ebyoebinanzijibwakobiterekebwaokweyambisibwa mu kunonyerezaokwebiseeraeby'omumaaso

....

Omujurizi;

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....

Omujuliziyetagisakw'abobokkaabakozesaekinkumu, obaabatasobolakusomalupapulalwabubakan'olwokukkrizzakw'abaana, obasingaomuntuafunaokukkirizzakw'omwanatayogeralulimilw'amwana .Omujurizitalinakubeeraomukubakozib'okunonyerezakunoobaomukubatuyambyeko mu kunonyerezanayeasobolaokubeeraomukub'omunnyumbaobaomukubakozib'eddwaliroatenyigira mu ntekatekazakunonyereza. Omujuriziatekwaokuberawo mu kiseerakyokuwaokukkirizakyona

Omuntuafunaokukkirizakw'omwana:

....

Erinnya (mu nnukutaennene) **Omukonoennakuz'omwezi**

Appendix 4: Information sheet for parents/guardians and consent form (English version)

Version 2.0 27th June 2014

Uganda Virus research Institute/Entebbe Hospital

Information parents/Guardian

Blood pressure study among 10 and 11 year old children in the Entebbe Mother and baby study

Introduction

Thank you for taking part in the earlier studies in the Mother and Baby study (EMaBS). This has helped us to collect a lot of information about health of children and their mothers. We are always trying to find new ways to use it to improve people's health.

We now want to use the information that we have collected to help us to study blood pressure in children in developing countries. We will find out if birth weight and other things that happened in early life affect blood pressure in children.

What is blood pressure?

Blood pressure is determined by the amount of blood the heart pumps and the amount of resistance to blood flow in the arteries. The more blood the heart pumps and the narrower the arteries, the higher the blood pressure. A person can have high blood pressure (hypertension) for a long time without any symptom. Uncontrolled high blood pressure increases the chances of serious health problems including death. A child with high blood pressure is likely to have hypertension when they are adults unless they begin treatment or alter their diet and life style.

Why is this study being done?

Hypertension is fast growing problem in developing countries as people change from a rural to an urban life style. Factors that influence blood pressure have been greatly studied in the developed countries. However there is little or no information from developing tropical countries. EMaBS children can help us to find outwhich factors are associated with high blood pressure among children in Uganda. This will help the Ministry of Health to plan treatment and management of children with hypertension, and the prevention of hypertension in adults.

Who is doing the study?

The EMaBS togetherwith Entebbe Hospital are carrying out this study and it will end in 2016.

Why have you chosen my child?

We have chosen your child because she/he is 10 or 11 years of age and under follow up in the EMaBS. We have collected a lot of information about her/his health from they were in the mother's womb to date. We think that this information togetherinformation about the child's blood pressure will help usanswers questions about blood pressure in children from developing countries.

1

What will happen if I allow my child to take part?

Some EMaBS activities will continue, just as before:

- The child will be seen,ask questions about their health and height and weightmeasurements will be taken when you are 10 or 11 years old.
- Your child will provide a stool sample.
- After giving a stool sample, your child willreceive treatment for worms. If the results show that you need special treatment, one of the fieldworkers will come and bring the treatment to your home.

If you agree to your child to take part in this additional study about blood pressure when they are 10 or 11 years of age, this is what willhappen.

- As well as height and weight, we will measure the size of your child's waist. We will then take their blood pressure and this will be done three times on the first day. Quite often the reading is high on the first day, just because the child is not used to having their blood pressure measured. If it is high on the first day we will invite you to come back so that the blood pressure reading can be taken again on a second and third day.
- The results of your child blood pressure will be compared to the normal values for his/ her age, sex and height.
- A blood sample (14mls, about three smallteaspoons) will be taken.
- Your child will provide a urine sample that will be stored .
- We will combine this information with data we already have about your child including information about your child's genes. We will look for particular genes that may affect blood pressure and other diseases related to your child's heart and blood vessels.
- The transport cost will be refunded. The amount given will depend on where you stay.
- If found with abnormal values of blood pressure on day 3, your child will be investigated further in consultation with a specialist doctor at Mulago Hospital. You will be advised about ways in which the blood pressure can be improved through change in lifestyle, and treatment will be provided if it is required.

What will the stool and blood samples be used for?

The stool and blood samples will be tested for infections. The blood sample will also be tested for chemical changes such as sugar and lipid (fat) levels: high sugar levels and abnormal lipid levels quite often occur along sideproblems with blood pressure. Some of the blood sample, and the urine sample, will be stored for other tests in the future, including measurement of substances involved in the immune system and nutrition. All the information collected will be linked to information that we already have, including the information about child's genes. This information and results of the test will all be kept confidentially and accessible only for research and to help in treatment of your child. The results of

the sugar and lipid levels will be available to you and if they are not normal you will be advised what to do.

Will this cause any problem to my child?

Taking to part in this study is not expected to cause any problems to your child. Measuring blood pressure and taking a blood sample may be uncomfortable to the child. But this is temporary and not expected not to have any danger to your child.

Are there any benefits to taking part in the study?

Because high blood pressure can occur without causing any symptoms, it is likely to be beneficial for your child to have this test done. It is a normal part of good, routine medical care. Results of the blood tests will also be useful for your child's health.

There is a very small possibility that we may discover some genes that increase the risk of your child having high blood pressure or related diseases which may have implications for their health or the health of their children. If we discover that your child has such genes we will make every effort to contact you or your child and provide appropriate guidance with what to do. We think the chances of this happening are very small.

It is your right to refuse or withdraw from the study.

Taking part in this study is voluntary. This means that you or the child can say that you do not what to be involved in the study. You can change your mind about your child's taking part in the study at any time. If you do not wish your child to take part in this study you may still continue under follow up within the EMaBS programme.

You can find out more about this study at any time by asking one of the study doctors, nurses or field workers at the clinic. You can also contact Dr. Elliott (telephone: 0417704000) or the Medical Superintendent of Entebbe Hospital (telephone: 320058), or the Ethics Committee Chairman from Uganda Virus Research Institute on 0414 321962.

Version 2.0 27th June 2014

Uganda Virus research Institute/Entebbe Hospital

Consent form for parent or guardian

Blood pressure study among 10 and 11 year old children in the Entebbe Mother and baby study

Child's name..... ..... Child's BIDNO |___|___|___|___|/|___|

My signature below shows that I have read and/or been fully explained the information sheet concerning my child's participation in this study and I understand what will be required if they agree to take part in the study.My questions concerning this study have been answered.My child's participation is voluntary and agrees to participant in this study.

Parent/Guardian's signatures:

1. My signature/thumb print indicates that I agree to have my child take part in the blood pressure study.
2. Relationship to the child .....

Name (Block Capitals) **Signature** or **thumb print** **Date**

....

3. My signature/thumb print indicates that I agree for part of the specimens to be stored for future studies

....

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Witness;

....

Witness required only for those using a thumb print, or unable to read the information and assent form, or if the person taking the assent does not speak the participant's language. The witness must not be a member of the research team or a study participant but may be a family member or a member of hospital staff who is not involved in the research programme. The witness much be present for the whole consent process.

Person conducting the consent:

.....

Name (Block Capitals) **Signature** **Date**

Appendix 5: Information sheet for parents/guardians and consent form (Luganda version)

Version 2.0 27th June 2014

Uganda Virus research Institute/Entebbe Hospital

Obubaka bw'abazadde/abakuza

Okunnonyereza kw'entununsi mu baana eb'emyaka ekkumi (10) n'ekkumi n'ogumu (11) mu kibiina kya ba maama n'abaana ekya Entebbe Mother and Baby Study (EMaBS).

Ennyanjula

Webale kwenyigira mu kunonyereza okwasooka okwa EMaBS. Kino kituyambye okukunganya amawulire mangi agakwata ku bulamu bw'abaana era ne bamaama babwe. Tugezaako buli kadde okuzuula engeri empya ey'okugeyambisa okulongoosa obulamu bwa'abantu.

Kati twagala kweyambisa amawulire getukunganyizza okutuyamba okunonyereza ku ntunnunsi mu baana mu nsi ezikyakula. Tujja kuzuula oba obuzito bw'omwana eyakazalibwa wamu n'ebintu ebirara ebiberawo munnaku ezisooka mu bulamu bikosa entunnunsi mu baana.

Entunnunsi kye ki?

Entunnunsi zikolebwa ekipimo ky'omusaayi omutima gwe gusunda wamu n'ekipimo ky'obukakanyavu bw'entambula y'omusaayi mu misuwa egijja omusaayi ku mutima. Omusaayi gyegukoma obungi omutima gwegusunda era n'emisuwa egijja omusaayi ku mutima gye gikoma obufunda, n'entunnunsi gyezikoma okweyongera. Omuntu ayinza okuba n'entunnunsi eziri waggulu w'ekipimo ekituufu okumalira ddala ekiseera kiwanvu nga talina kabonero konna. Entunnunsi ezisusse ekipimo nga tezifiriddwako zongeza emikisa gy'okufuna ebizibu eby'amanyi ku bulamu nga mwotwalidde n'okufa. Omwana alina entunnunsi ezisusse ekipimo ayinza okufuna obulwadde bw'entunnunsi ng'akuze okujjako ng'atandise eddagla oba ng'akyusizza mu by'okulya n'embera z'obulamu.

Lwaki okunonyereza kuno kukolebwa?

Obulwadde bw'entunnunsi bweyongedde nnyo mu nsi ezikyakula ng'abantu bakyusa okuva mu mbeera ez'ekyalo okudda ku z'ekibuga. Ebintu ebireeta entunnunsi binonyerezaddwako nnyo mu mawanga agaakula edda munsi. Wabula, waliwo amawulire matono ddala oba obutabawo n'akamu munsi ezikyakula. Abaana ba EMaBS bayinza okutuyamba okuzuula biki ebyekuusa ku ntunnunsi ezisusse ekipimo mu baana mu Uganda. Kino kijja kuyamba ekitongole ky'eby'obulamu okuteekateeka obujjanjabi n'okulabirira abaana abalina obulwadde bw'entunnunsi, n'okuziyiza obulwadde bw'entunnunsi mu bantu abakulu.

Ani akola okunonyereza?

Ekibiina kya EMaBS wamu n'eddwaliro ly'Entebbe era kujja kuggwa mu 2016.

Lwaki mulonze omwana wange?

Tulonze omwana wo kubanga alina emyaka Kkumi (10) oba kkumi nagumu (11) era nga ali mu kibiina kya EMaBS. Tukunganyizza amawulire mangi agakwata ku bulamubwe okuva nga akyali mu lubuuto lwa

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maama we okutuusa kati. Tulowooza nti amawulire gano, wamu n'ago agakwata ku ntunnunsi z'omwawo gajja kutuyamba okwanukula ebibuuzo ku bulwadde bw'entunnunsi mu baana mu mawanga agakyakula.

Kiki ekinabawo singa nzikiriza omwana wange okwetabamu?

Ebimu ku bikolebwa mu EMaBS bijja kweyongera, era nga bulijjo:

- Omwana ajja kulabibwa, abuuze ebibuuzo ebikwata ku bulamu bwe, era apimibwe obuwanvu n'obuzito
- Omwawo ajja kuwayo ekyolooni.
- Omwanawo bwanamala okuwayo ekyolooni ajja kufuna eddagala ly'ebiwuka by'omulubuto. Singa ebiva mu kukebera bilaga nti yetaaga obujjanjabi obw'enjawulo, omu ku bakozi (abasawo) baffe ajja kuleeta obujjanjabi awaka wamwe.

Singa okkiriza omwanawo okwetaba mu kunononyereza kuno okwongedwaddeko okw'obulwadde bw'entunnunsi nga wa myaka kkumi(10) oba kkumi nagumu, bino bye bijja okubaawo.

- Nga obuwanvu n'obuzito, tujja kupima ekiwato ky'omwanawo. Tujja era kupima entunnunsi ze, era kino kijja kukolebwa emirundi esatu (3) ku lunaku olusooka. Emirundi egisinga ebipimoby'okw'olunaku olusooka bibeera wagula, lwakuba omwana abeera tamanyidde kumupima ntunnunsi. Bwebiba waggulu ku lunaku olusooka tujja ku bayita mukomewo tuddemu tupimeku lunaku olw'okubiri era n'olw'okusatu.
- Ebivudde mu kupima omwanawoentunnunsi bijja kugerageranyizibwa ku bipimo ebyabulijjo eby'emyaka gye, ekikula era n'obuwanvu bwe.
- Tujja kukujjako sampolo y'omusaayi obujiiko bwa kyayi obutuno nga busattu(3) (14mls).
- Sampolo y'omusulo ejja kukujjibwako eterekebwe.
- Tujja kugatta amawulire gano gonna wamu n'ago getukungayizza agakwata ku mwana wo okuva nga yakazaalibwa nga kwotadde n'ebyo ebikwata ku buzalilanwabwe obw'endagabutonde. Tujja kunonya endagabutondo ezo ezlyinza okutataganya entunnunsi n'endwadde endala ez'ekuusa ku mutima gw'omwana wo n'emisuwa.
- Sente z'entabula zijja kukuddizibwawo. Omuwendo gujja kusinziira wa gyova.
- Singa azulibwamu ebipimo ebitali bya bulijjo ku lunaku olw'okusatu, omwanawo ajja kwongera okwekebejjebwanga twebuuza ku musawo omukugu mu ddwaliro ly'eMulago. Ojja kuwebwa amagezi kungeri ki entununsi zino gyeziyinza okukendezebwa nga akyusa mu mbeera z'eby'obulamu, n'obujjanjabi bujja kumuwebwa singa kyetagisizza.

Sampolo z'ekyoloni n'omusaayi zakukozesa ki?

Sampolo z'ekyoloni n'omusaayi zijja kukeberebwamu endwadde. Sampolo y'omusaayi, ejja kukeberebwamuebintu nga ssukaali namasavu; ebipimo byassukaali ebiri wagulu namasavu amangi bitera okubeerawookumu n'ebizibu by'entunnunsi eziri wagulu. Sampolo z'omusaayi ezimu wamu n'omusulo zijja kuterekebwa zeyambisibwe mu kukebera okwenjawulo mu biseera eby'omu maaso, nga mwemuli okukebera ebintu ebirara nga amanyi g'omubiri n,ebyendiisa. Amawulire gonna

getukunganyiza gajja kugttibwaku ago getulina, nga mwemuli n'ago agakwata ku ndagabutonde z'omwana.

Amawulire gano nebinava mu kukebera bijja kukumibwa nga byakayama era nga byeyambisibwa mu kunonyereza kwokka n'okuyamba mu kujjanjaba omwanawo. Ebinava mu kukebera ssukaali n'obungi bw'amasavu bujja kukumibwa era singa tebibera birungi, ojja kuwebwa amagezi ku kiki eky'okukola.

Kino kinaleeta obuzibu bwonna ku mwana wange?

Okwetaba mu kunonyereza kuno tekisuubirwa kuleetewo buzibu bwonna ku mwana wo. Okupima entuununsi n'okukujjako sampolo z'omusaayi kiyinza okukalubirizamu omwana wo. Naye kino kya kaseera katono era nga tekisuubirwa kubeera nabuzibu bwonna ku mwana wo.

Waliwo emiganyulo gyonna mu kwetaba mukunonyereza kuno?

Olw'okubanga entunnunsi ziyinza okubeera wagulu nga tekireese kabonero konna, kiyinza okubeera ekyomugaso omwanawo okupimibwa. Kimu ku bitundu ebirungi mukujjanjabwa okwabulijjo. Ebinava mu kukebera kw'omusaayi nabyo bijja kubeera bya mugaso eri obulamu bw'omwana wo.

Waliwo okutebereza kutono ddala nti tuyinza okuzuula kundagabutonde eziyinza okwongera emikisa gy'omwanawo okuba n'entunnunsi oba endwadde ezekwanaganya ekiyinza okuleetawo obuzibu kubulamu bwe oba obw'abaana be. Singa tukizuula nti omwanawo alina endagabutonde ezekika ekyo, tujja kukola kyonna ekisoboka okukutuukirira oba omwanawo tubawe okulungamizibwa okusanidde ku kiki eky'okukola ekituufu. Tulowooza nti emikisa gya kino okubeerawo mitono ddala

Ddembe lyo okugaana oba okuva mu kunonyereza kuno.

Okwetaba mu kunonyereza kuno kwakyeyagalire. Kino kitegeeza nti ggwe oba omwanawo musobola okugamba nti temwagala kwetabamukunonyereza. Oyinza okukyusa mu ndowooza ku ky'omwanawo okwetaba mukunonyereza kuno ekiseera kyonna. Bwoba toyagala omwanawo yetabe mu kunonyereza kuno oyinza okweyongerayo n'entekateka za EMaBS eza bulijjo

Oyinza okumanya ebisingawo ku kunonyereza kuno ekiseera kyonna ng'obuuza omu ku basawo b'okunonyereza, ba nansi oba abakozi ku kilinika. Oyinza era okutukurira Dokita Elliott (essimu:0417704000) oba omukulu w'eddwaliro ly'Entebbe (Essimo 0414320058), oba omukulu w'akakiiko aka Sayansi n'empissa okuva mu Uganda Virus Research Institute ku ssimu 0414321961.

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Version 2.0 27th June 2014

Uganda Virus research Institute/Entebbe Hospital

Okukkiriza kw'omuzadde oba omukuza

Okunonyereza ku bulwadde b'entunnunsi mu baana ab'emyaka egigenda mu ssomero mu kibiina kya ba maama n'abaana (EMaBS)

Erinnya ly'omwana.....Ennamba y'omwana BIDNO |___|___|___|___|/|___|

Omukono gwange wamanga gulaga ntinsomye oba banyinnyonyoddemu bujjuvu olupapula lw'obubaka obukwata ku kwetaba kw'omwana wange mu kunonyereza kuno era ntegedde biki ebyetagisa singa akkiriza okwetaba mu kunonyereza.Ebibuuzo byange ebikwata ku kunonyereza kuno biddiddwamu. Okwetamu kw'omwana wange kwa kyeyagalire era akkiriza okwetaba mu kunonyereza kuno.

Emikono gy'omuzadde/omukuza:

1. Omukono gwange/ekinkumu kiraga nti nzikiriza omwana wange yetabe mu kunonyereza ku ntunnunsi
2. Oluganda ku mwana .....

Erinnya (mu nnukuta ennene) **Omukono** oba **ekinkumu** **ennaku z'omwezi**

....

3. Omukono gwange/ekinkumu kiraga nti nzikriza ebimu kubinajibwa ku mwana wange biterekebwe okweyambisibwa mu kunonyereza gyebujja mu maaso

....

Omujurizi;

....

Omujulizi yetagisa kw'abo bokka abakozesa ekinkumu, oba abatasobola kusoma lupapula lwabubaka n'olwokukkrizza kw'abaana, oba singa omuntu afuna okukkirizza kw'omwana tayogera lulimi lw'amwana . Omujulizi talina kubeera omu ku bakozi b'okunonyereza kuno kuno oba omu kubatuyabyeko mu kuunonyereza naye asobola okubeera omu ku b'omunnyumba oba omu ku bakozi b'eddwaliro atenyigira mu ntekateka za kunonyereza. Omujulizi atekwa okuberawo mu kiseera kyokuwa okukkiriza kyona

Omuntu afuna okukkiriza:

....

Erinnya (mu nnukuta ennene) **Omukonoennaku z'omwezi**

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Appendix 6: Blood pressure level for boys by age and height percentile

Age, y	BP Percentile	SBP, mm Hg							DBP, mm Hg					
			Percentile of Height							Percentile of Height				
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	80	81	83	85	87	88	89	34	35	36	37	38	39	39
	90th	94	95	97	99	100	102	103	49	50	51	52	53	53	54
	95th	98	99	101	103	104	106	106	54	54	55	56	57	58	58
	99th	105	106	108	110	112	113	114	61	62	63	64	65	66	66
2	50th	84	85	87	88	90	92	92	39	40	41	42	43	44	44
	90th	97	99	100	102	104	105	106	54	55	56	57	58	58	59
	95th	101	102	104	106	108	109	110	59	59	60	61	62	63	63
	99th	109	110	111	113	115	117	117	66	67	68	69	70	71	71
3	50th	86	87	89	91	93	94	95	44	44	45	46	47	48	48
	90th	100	101	103	105	107	108	109	59	59	60	61	62	63	63
	95th	104	105	107	109	110	112	113	63	63	64	65	66	67	67
	99th	111	112	114	116	118	119	120	71	71	72	73	74	75	75
4	50th	88	89	91	93	95	96	97	47	48	49	50	51	51	52
	90th	102	103	105	107	109	110	111	62	63	64	65	66	66	67
	95th	106	107	109	111	112	114	115	66	67	68	69	70	71	71
	99th	113	114	116	118	120	121	122	74	75	76	77	78	78	79
5	50th	90	91	93	95	96	98	98	50	51	52	53	54	55	55
	90th	104	105	106	108	110	111	112	65	66	67	68	69	69	70
	95th	108	109	110	112	114	115	116	69	70	71	72	73	74	74
	99th	115	116	118	120	121	123	123	77	78	79	80	81	81	82
6	50th	91	92	94	96	98	99	100	53	53	54	55	56	57	57
	90th	105	106	108	110	111	113	113	68	68	69	70	71	72	72
	95th	109	110	112	114	115	117	117	72	72	73	74	75	76	76
	99th	116	117	119	121	123	124	125	80	80	81	82	83	84	84
7	50th	92	94	95	97	99	100	101	55	55	56	57	58	59	59
	90th	106	107	109	111	113	114	115	70	70	71	72	73	74	74
	95th	110	111	113	115	117	118	119	74	74	75	76	77	78	78
	99th	117	118	120	122	124	125	126	82	82	83	84	85	86	86
8	50th	94	95	97	99	100	102	102	56	57	58	59	60	60	61
	90th	107	109	110	112	114	115	116	71	72	72	73	74	75	76
	95th	111	112	114	116	118	119	120	75	76	77	78	79	79	80
	99th	119	120	122	123	125	127	127	83	84	85	86	87	87	88
9	50th	95	96	98	100	102	103	104	57	58	59	60	61	61	62
	90th	109	110	112	114	115	117	118	72	73	74	75	76	76	77
	95th	113	114	116	118	119	121	121	76	77	78	79	80	81	81
	99th	120	121	123	125	127	128	129	84	85	86	87	88	88	89
10	50th	97	98	100	102	103	105	106	58	59	60	61	61	62	63
	90th	111	112	114	115	117	119	119	73	73	74	75	76	77	78
	95th	115	116	117	119	121	122	123	77	78	79	80	81	81	82
	99th	122	123	125	127	128	130	130	85	86	86	88	88	89	90
11	50th	99	100	102	104	105	107	107	59	59	60	61	62	63	63
	90th	113	114	115	117	119	120	121	74	74	75	76	77	78	78
	95th	117	118	119	121	123	124	125	78	78	79	80	81	82	82
	99th	124	125	127	129	130	132	132	86	86	87	88	89	90	90
12	50th	101	102	104	106	108	109	110	59	60	61	62	63	63	64
	90th	115	116	118	120	121	123	123	74	75	75	76	77	78	79
	95th	119	120	122	123	125	127	127	78	79	80	81	82	82	83
	99th	126	127	129	131	133	134	135	86	87	88	89	90	90	91
13	50th	104	105	106	108	110	111	112	60	60	61	62	63	64	64
	90th	117	118	120	122	124	125	126	75	75	76	77	78	79	79
	95th	121	122	124	126	128	129	130	79	79	80	81	82	83	83
	99th	128	130	131	133	135	136	137	87	87	88	89	90	91	91
14	50th	106	107	109	111	113	114	115	60	61	62	63	64	65	65
	90th	120	121	123	125	126	128	128	75	76	77	78	79	79	80
	95th	124	125	127	128	130	132	132	80	80	81	82	83	84	84
	99th	131	132	134	136	138	139	140	87	88	89	90	91	92	92
15	50th	109	110	112	113	115	117	117	61	62	63	64	65	66	66
	90th	122	124	125	127	129	130	131	76	77	78	79	80	80	81
	95th	126	127	129	131	133	134	135	81	81	82	83	84	85	85
	99th	134	135	136	138	140	142	142	88	89	90	91	92	93	93
16	50th	111	112	114	116	118	119	120	63	63	64	65	66	67	67
	90th	125	126	128	130	131	133	134	78	78	79	80	81	82	82
	95th	129	130	132	134	135	137	137	82	83	83	84	85	86	87
	99th	136	137	139	141	143	144	145	90	90	91	92	93	94	94
17	50th	114	115	116	118	120	121	122	65	66	66	67	68	69	70
	90th	127	128	130	132	134	135	136	80	80	81	82	83	84	84
	95th	131	132	134	136	138	139	140	84	85	86	87	87	88	89
	99th	139	140	141	143	145	146	147	92	93	93	94	95	96	97

The 90th percentile is 1.28 SD, the 95th percentile is 1.645 SD, and the 99th percentile is 2.326 SD over the mean.
For research purposes, the SDs in Table B1 allow one to compute BP Z scores and percentiles for boys with height percentiles given in Table 3 (ie, the 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles). These height percentiles must be converted to height Z scores given by: 5% = −1.645; 10% = −1.28; 25% = −0.68; 50% = 0; 75% = 0.68; 90% = 1.28; and 95% = 1.645, and then computed according to the methodology in steps 2 through 4 described in Appendix B. For children with height percentiles other than these, follow steps 1 through 4 as described in Appendix B.

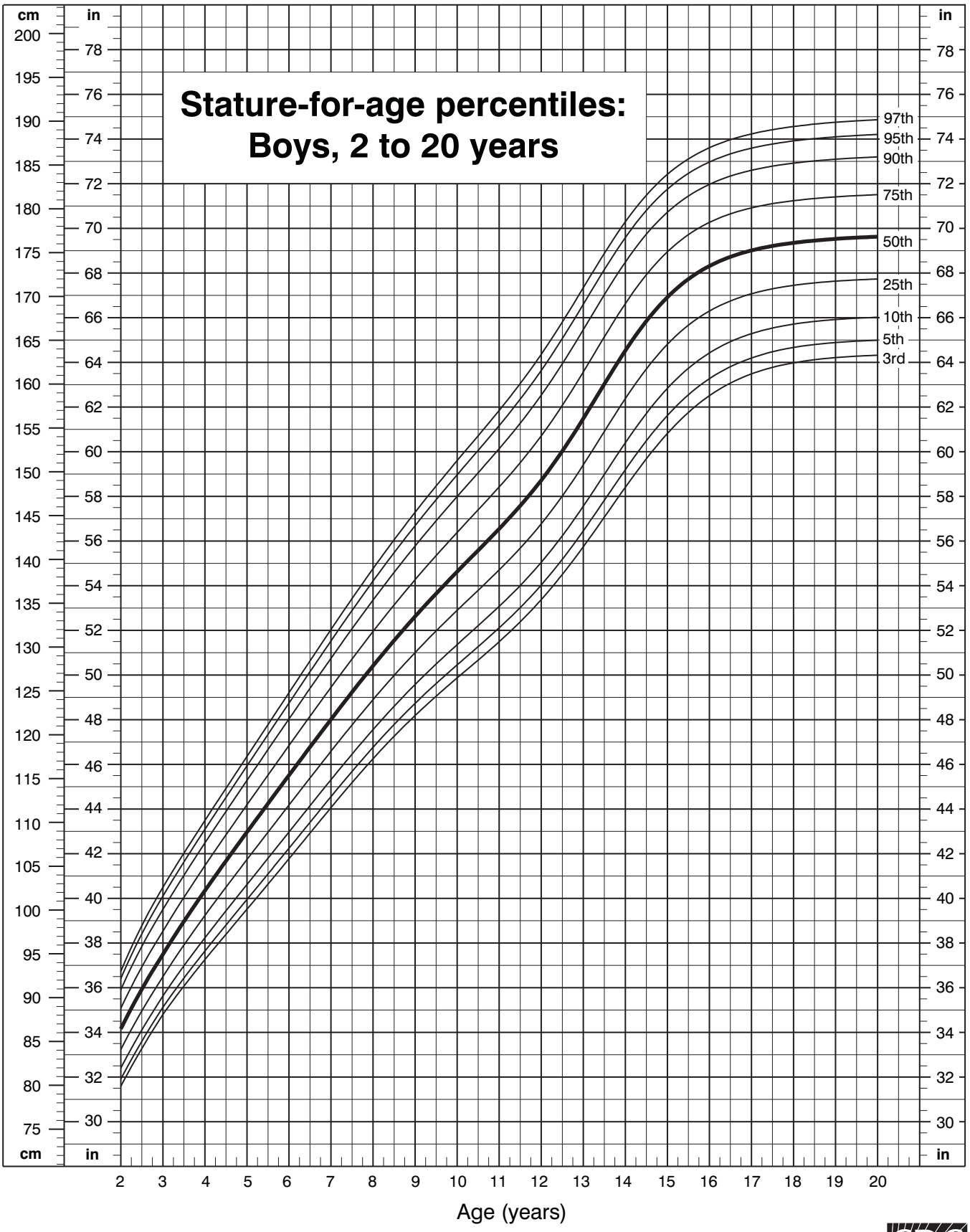
Appendix 7: Blood pressure level for girls by age and height percentile

Age, y	BP Percentile	SBP, mm Hg							DBP, mm Hg						
			Percentile of Height							Percentile of Height					
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	83	84	85	86	88	89	90	38	39	39	40	41	41	42
	90th	97	97	98	100	101	102	103	52	53	53	54	55	55	56
	95th	100	101	102	104	105	106	107	56	57	57	58	59	59	60
	99th	108	108	109	111	112	113	114	64	64	65	65	66	67	67
2	50th	85	85	87	88	89	91	91	43	44	44	45	46	46	47
	90th	98	99	100	101	103	104	105	57	58	58	59	60	61	61
	95th	102	103	104	105	107	108	109	61	62	62	63	64	65	65
	99th	109	110	111	112	114	115	116	69	69	70	70	71	72	72
3	50th	86	87	88	89	91	92	93	47	48	48	49	50	50	51
	90th	100	100	102	103	104	106	106	61	62	62	63	64	64	65
	95th	104	104	105	107	108	109	110	65	66	66	67	68	68	69
	99th	111	111	113	114	115	116	117	73	73	74	74	75	76	76
4	50th	88	88	90	91	92	94	94	50	50	51	52	52	53	54
	90th	101	102	103	104	106	107	108	64	64	65	66	67	67	68
	95th	105	106	107	108	110	111	112	68	68	69	70	71	71	72
	99th	112	113	114	115	117	118	119	76	76	76	77	78	79	79
5	50th	89	90	91	93	94	95	96	52	53	53	54	55	55	56
	90th	103	103	105	106	107	109	109	66	67	67	68	69	69	70
	95th	107	107	108	110	111	112	113	70	71	71	72	73	73	74
	99th	114	114	116	117	118	120	120	78	78	79	79	80	81	81
6	50th	91	92	93	94	96	97	98	54	54	55	56	56	57	58
	90th	104	105	106	108	109	110	111	68	68	69	70	70	71	72
	95th	108	109	110	111	113	114	115	72	72	73	74	74	75	76
	99th	115	116	117	119	120	121	122	80	80	80	81	82	83	83
7	50th	93	93	95	96	97	99	99	55	56	56	57	58	58	59
	90th	106	107	108	109	111	112	113	69	70	70	71	72	72	73
	95th	110	111	112	113	115	116	116	73	74	74	75	76	76	77
	99th	117	118	119	120	122	123	124	81	81	82	82	83	84	84
8	50th	95	95	96	98	99	100	101	57	57	57	58	59	60	60
	90th	108	109	110	111	113	114	114	71	71	71	72	73	74	74
	95th	112	112	114	115	116	118	118	75	75	75	76	77	78	78
	99th	119	120	121	122	123	125	125	82	82	83	83	84	85	86
9	50th	96	97	98	100	101	102	103	58	58	58	59	60	61	61
	90th	110	110	112	113	114	116	116	72	72	72	73	74	75	75
	95th	114	114	115	117	118	119	120	76	76	76	77	78	79	79
	99th	121	121	123	124	125	127	127	83	83	84	84	85	86	87
10	50th	98	99	100	102	103	104	105	59	59	59	60	61	62	62
	90th	112	112	114	115	116	118	118	73	73	73	74	75	76	76
	95th	116	116	117	119	120	121	122	77	77	77	78	79	80	80
	99th	123	123	125	126	127	129	129	84	84	85	86	86	87	88
11	50th	100	101	102	103	105	106	107	60	60	60	61	62	63	63
	90th	114	114	116	117	118	119	120	74	74	74	75	76	77	77
	95th	118	118	119	121	122	123	124	78	78	78	79	80	81	81
	99th	125	125	126	128	129	130	131	85	85	86	87	87	88	89
12	50th	102	103	104	105	107	108	109	61	61	61	62	63	64	64
	90th	116	116	117	119	120	121	122	75	75	75	76	77	78	78
	95th	119	120	121	123	124	125	126	79	79	79	80	81	82	82
	99th	127	127	128	130	131	132	133	86	86	87	88	88	89	90
13	50th	104	105	106	107	109	110	110	62	62	62	63	64	65	65
	90th	117	118	119	121	122	123	124	76	76	76	77	78	79	79
	95th	121	122	123	124	126	127	128	80	80	80	81	82	83	83
	99th	128	129	130	132	133	134	135	87	87	88	89	89	90	91
14	50th	106	106	107	109	110	111	112	63	63	63	64	65	66	66
	90th	119	120	121	122	124	125	125	77	77	77	78	79	80	80
	95th	123	123	125	126	127	129	129	81	81	81	82	83	84	84
	99th	130	131	132	133	135	136	136	88	88	89	90	90	91	92
15	50th	107	108	109	110	111	113	113	64	64	64	65	66	67	67
	90th	120	121	122	123	125	126	127	78	78	78	79	80	81	81
	95th	124	125	126	127	129	130	131	82	82	82	83	84	85	85
	99th	131	132	133	134	136	137	138	89	89	90	91	91	92	93
16	50th	108	108	110	111	112	114	114	64	64	65	66	66	67	68
	90th	121	122	123	124	126	127	128	78	78	79	80	81	81	82
	95th	125	126	127	128	130	131	132	82	82	83	84	85	85	86
	99th	132	133	134	135	137	138	139	90	90	90	91	92	93	93
17	50th	108	109	110	111	113	114	115	64	65	65	66	67	67	68
	90th	122	122	123	125	126	127	128	78	79	79	80	81	81	82
	95th	125	126	127	129	130	131	132	82	83	83	84	85	85	86
	99th	133	133	134	136	137	138	139	90	90	91	91	92	93	93

* The 90th percentile is 1.28 SD, the 95th percentile is 1.645 SD, and the 99th percentile is 2.326 SD over the mean.

For research purposes, the SDs in Table B1 allow one to compute BP Z scores and percentiles for girls with height percentiles given in Table 4 (ie, the 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles). These height percentiles must be converted to height Z scores given by: 5% = −1.645; 10% = −1.28; 25% = −0.68; 50% = 0; 75% = 0.68; 90% = 1.28; and 95% = 1.645 and then computed according to the methodology in steps 2 through 4 described in Appendix B. For children with height percentiles other than these, follow steps 1 through 4 as described in Appendix B.

Appendix 8: Centers for Disease Control and Prevention growth charts



Published May 30, 2000.
SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).

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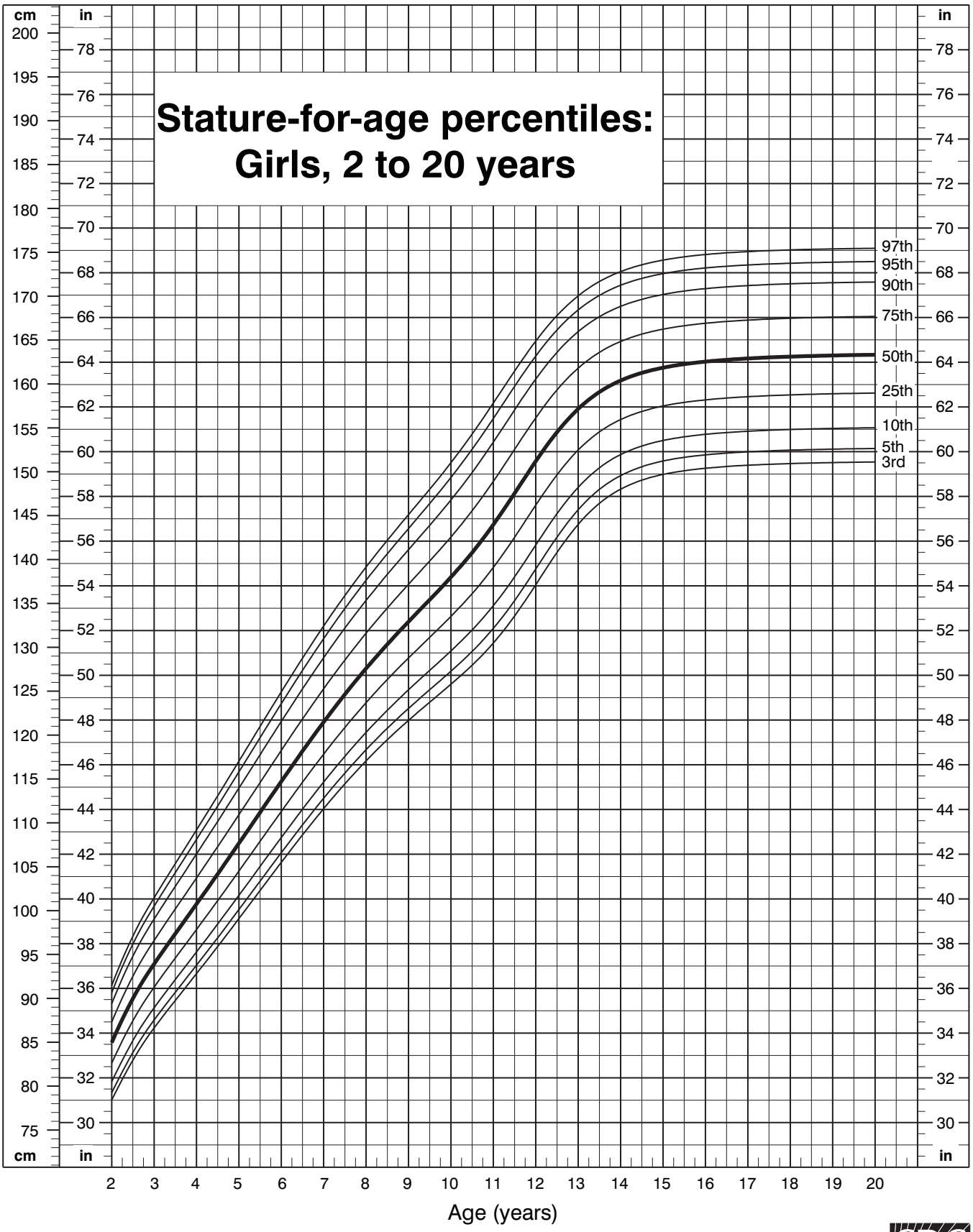

Published May 30, 2000.

SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).

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Appendix 9: Blood pressure study questionnaire at 10 and 11 years of age

BPSVISIT, Version 2.0 06/03/15 Child's ID number |___|___|___|___|/|___| **BIDNO**

BLOOD PRESSURE STUDY VISIT, AT TEN or ELEVEN YEARS OF AGE

NAME

Child's Name: …….....

Date of visit (dd/mm/201y) |__|__|/|__|__|/| 2 | 0 | 1 |__| **DATEVB1**

1 **What is the child's age on this visit?** |__|__| **YEARS** |__|__| **MONTHS**

2 **When did the child last have meal** *(Meal includes food or any drink other than water)*

Time (in 24hrs) |__|__| . |__|__| **MEALTM**

Date (dd/mm/201y) |__|__|/|__|__|/| 2 | 0 |1 |__| **MEALDT**

Time of blood collection

Time (in 24hrs) |__|__| . |__|__| **BLODTM**

Date (dd/mm/201y) |__|__|/|__|__|/| 2 | 0 |1 |__| **BLODT**

Anthropometric Measurements

3 **Weight** |__|__|.|__| kg **WEIGHT**

4 **Height** |__|__|__|.|___| cm **HEIGHT**

5 **Height percentile for child's sex and age** |__|__| th **HTPER**

Waist circumference and Mid upper arm circumference measurements (cm).

6 waist circumference |__|__|.|__| cm **WSRT** mid upper arm circumference |__|__|.|__| cm **ARM**

For Girls only

7 **Has the child started having menses?** (1=yes, 2=no, 9=don't know; if there is uncertainty use "don't know") |___| **MENS**

Information about the child and the family.

Interviewer please explain that, although we do have a lot of information about our children, we are taking a special interest in factors that may be related to blood pressure at this visit and so will ask some additional questions and may repeat some that we have already asked at earlier visits.

Child and Family history

8 **Has the child ever had blood pressure measured by a professional health worker?** (1=yes, 2=no, 9=don't know; if there is uncertainty use "don't know") |___| **PRES**

9 **Has the child ever been told by a doctor or health worker that they have raised blood pressure?** (1=yes, 2=no, 9=don't know; if there is uncertainty use "don't know") |___| **HYPC**

10 *If yes,* **Is he/she on treatment for high blood pressures?** (1=yes, 2=no, 9=don't know; if there is uncertainty use "don't know") |___| **HYPT**

11 **Does the child have a close relative (father, mother, brother or sister) who has ever been diagnosed by a doctor or nurse with any of the following conditions?** (1=yes, 2=no, 9=don't know; if there is uncertainty use "don't know")

High blood pressure |___| **HBP**

Heart attack / angina (chest pain on exertion or walking) |___| **HART**

Stroke |___| **SKRO**

Diabetes |___| **DIAB**

12 **Does the child stay in a household in which someone smokes cigarettes in the home?** (1=yes, 2=no, 9=don't know; if there is uncertainty use "don't know") |___| **SMOK**

	Sleeping pattern		
13	**Does your child have any problems at bedtime or going to bed?** (1=yes, 2=no, 9=don't know; if there is uncertainty use "don't know")	|___|	**BEDT**
14	**Does your child have difficulty waking in the morning, seem sleepy during the day** (1=yes, 2=no, 9=don't know; if there is uncertainty use "don't know")	|___|	**NAP**
15	**Does your child often take naps during the day?** (1=yes, 2=no, 9=don't know; if there is uncertainty use "don't know")	|___|	**DNAPS**
16	*If yes child has nap,* **for how long on average is the nap***?* (enter time in minutes)	|___|___|___|	**NAPTM**
17	**Does your child seem to wake up a lot at night? Any sleep waking or nightmares?** (Answer 1= yes, 2=No and 9= uncertain)	|___|	**WAKE**
18	**On average, how many times does your child wake up at night? (**Answer 99 if you do not know)	|___|___|	**WAKE**
19	**Do you think this child gets enough sleep?** (1=yes, 2=no, 9=don't know; if there is uncertainty use "don't know")	|___|	**ENOU**
20	**On average, for how many hours is the child's sleep during the night time?** (enter time in hours)	|___|___|	**SLEEP**
21	**Does your child have loud or nightly snoring or any breathing difficulty at night?** (1=yes, 2=no, 9=don't know; if there is uncertainty use "don't know")	|___|	**SNORE**
22	**Does the child have brief spells in which he or she stops breathing during his or her sleep?** (1=yes, 2=no, 9=don't know; if there is uncertainty use "don't know")	|___|	**APNEA**
	Diet		
23	**In the past one month, in a typical week, on how many days does the child eat fruit, such as pineapple, mango, jackfruit, apples etc?** (enter number of days per week 0-7; enter 9 if not sure)	|___|	**DIET1**
24	**In the past one month, in a typical week, on how many days does the child eat vegetables, such cabbage, avocado, greens, carrots, and eggplants etc?** (enter number of days per week 0-7; enter 9 if not sure)	|___|	**DIET2**
25	**In the past one month, in a typical week, on how many days does the child drink sugared drinks like soda, juices?** (enter number of days per week 0-7; enter 9 if not sure)	|___|	**DIET3**
26	**In the past one month, in a typical week, did the child add salt to your food in addition to what has been used when cooking?** (1=yes 2=no; enter 9 if not sure)	|___|	**DIET4**
27	**In the past one month, in a typical week, can you estimate how many (total) teaspoons (small spoons) of sugar is normally added to the child's cup of tea or any other drink you take?** (enter 99 if not sure)	|___|___|	**DIET5**
28	**In the past one month, in a typical week, on how many days does the child eat starchy staples foods, such as posho, cassava, rice, sweet potatoes, bananas/plantains, etc?** (enter number of days per week 0-7; enter 9 if not sure)	|___|	**DIET 6**
29	**In the past one month, in a typical week, on how many days do you eat beef/goat meat/pork/mutton, fish, chicken or other animal proteins?** (enter number of days per week 0-7; enter 9 if not sure)	|___|	**DIET7**
30	**What type of oil or fat is most used for food preparation in your household (**answer **1=vegetable oil** (e.g. kimbo, cowboy, sunseed oil, olive oil, fortune oil, roki oil, mukwano oil), **2=animal oil** (e.g Ghee, butter, lard), **3=margarine** (e.g. Blue Band)**, 4= none in particular, 5 none used, 6=other, 9= I do not know**	|___|	**OIL**
	If entered 6 (Others) **specify the oil or fat type used** ……. **OILTH**		
	Breast feeding History		
31	**Was this child breastfed during infancy?** (Answer 1= yes, 2=No and 9= uncertain)	|___|	**BFED**
32	**How old was the children when extra feeds (other than drinking water and breast milk) introduced? (**in months, answer 99=uncertain**)**	|___|___|	**EFED**
33	**How old was the child when breast feeding was stopped? (**in months, answer 99=uncertain**)**	|___|___|	**SBFD**
	Physical Activity		

We are trying to find out about your level of physical activity This includes play, work or dance that make you sweat or

make you your legs feel tired, or games that make you breathe hard, like tag, skipping, running, climbing and others.

When at school

34	**Do you go to school? (1**=yes, **2**=no) If answer is **no**, then move to section **when at home**	|___|	**SCH**
35	**Are you a day scholar or a boarder (1=day, 2=boarder)**	|___|	**SCHTP**
36	**For a child who walks to school, how long does it take to get to school?** Answer in minutes: for boarders enter zero (0)	|__|__|__|	**TAK**
37	**Does your school have time allocated to physical activity (lessons and sport time (including sports or dancing))?** (Answer 1=yes, 2=no) If answer is **no**, then move to (question 39)	|___|	**PESCH**
38	**Do you participate in these physical activity/lessons (including sports and dancing)?** 1=yes, 2=no,	|___|	**LKPE**
39	**In a typical school week, for how long in total does the physical activity/lesson last per week? (including sports and dancing)** (Time in minutes)?	|__|__|__|	**MNPE**
40	**Do you have break times (at break or lunch periods) from class when you get involved in exercise (e.g.** Playing/skipping)? (Answer 1=yes, 2=no)	|__|	**BREAK**
41	**How much time in total do you spend playing during these beaks (break and lunch) per day?** (Time in minutes)?	|__|__|__|	**BREPL**

42 **What do you usually do after school each day?** (answer 1=yes, 2=no for each of the following)

If answer is **Yes, for how long?** (In minutes).

Play	|___|	**PLAY1**	|__|__|__|	**PLAYH1**
Reading	|___|	**READ1**	|__|__|__|	**READH1**
Watch Television	|___|	**TV1**	|__|__|__|	**TVH1**
Fatching water	|___|	**WAT1**	|__|__|__|	**WATH1**
Dance	|___|	**DANC1**	|__|__|__|	**DANCH1**
Digging	|___|	**DIG1**	|__|__|__|	**DIGH1**
Video game	|___|	**VIG1**	|__|__|__|	**VIGHS1**
Others	|___|	**OTS1**	|__|__|__|	**OTSH1**
Others	|___|	**OTY1**	|__|__|__|	**OTYH1**

When Home (the days when not going to school)

43 **When at Home (Weekend or Holiday or not going to school), What activities do you usually do and how much time per day do you usually spend on that activity** (1=Yes, 2=No, 3=Uncertain)

Play	|___|	**PLAY2**	|__|__|__|	**PLAYH2**
Reading	|___|	**READ2**	|__|__|__|	**READH2**
Watch Television	|___|	**TV2**	|__|__|__|	**TVH2**
Fatching water	|___|	**WAT2**	|__|__|__|	**WATH2**
Dance	|___|	**DANC2**	|__|__|__|	**DANCH2**
Digging	|___|	**DIG2**	|__|__|__|	**DIGH2**
Video game	|___|	**VIG2**	|__|__|__|	**VIGHS2**
Others	|___|	**OTS2**	|__|__|__|	**OTSH2**
Others	|___|	**OTY2**	|__|__|__|	**OTYH2**

Chronic illnesses

44 **Has the child ever been told by a doctor or other health worker that they suffered from any of the following chronic diseases?** (1=Yes, 2=No, 3=Uncertain)

Diabetes	|___|	**DIAT**
Heart condition	|___|	**HRTD**

Cancer	|___|	**CANC**
Sickle cell disease	|___|	**SCD**
HIV infection	|___|	**HIV**
Kidney disease	|___|	**KIDN**
Thyroid disease	|___|	**TYRO**

Drug use history

45 **Has the child had daily prolonged use of any of the following drugs (**indicate **1=**yes, **2=**no, **9=**not sure**)**

Antiretroviral drugs (ARVS)	|___|	**ARVS**
NSAID (e.g. ibuprofen, naproxen, cyclo0xagenase-2 inhibitors)	|___|	**NSAID**
Steroids (e.g. methylprednisolone, predislone)	|___|	**STEROIDS**
Psychiatric drugs (buspirone, carbamezepine, clozapine fluoxetine)	|___|	**PSYCHIA**
Sympathomimetics (Nasal decongestants, diet pills)	|___|	**SYMPAT**
Herbals	|___|	**HERBAL**
Others (mention .....)	|___|	**MENTION1**
Others (mention .....)	|___|	**MENTION2**

Tanner Stage (use the tanner stage charts)

For the male and female participants

What is the stage in public hair development? (answer 1=stage 1, 2= stage 11, 3=stage 111 and 4= stage 1V and 5=stage V) |___| **STAGPB**

For the Females (girls) participants only

What is the stage for the breast development? (answer 1=stage 1, 2= stage 11, 3=stage 111 and 4= stage 1V and 5=stage V) |___| **STAGBR**

DAY ONE

Blood pressure and pulse: systolic/diastolic blood pressure (mmHg) and pulse (beats/minute)

Take 3 reading, waiting 5 minutes between each reading

1st Systolic BP |__|__|__| **SBP1**	1ST Diastolic |__|__|__| **DBP1**	1st Pulse rate |__|__|__| **PRA1**
2nd Systolic BP |__|__|__| **SBP2**	2nd Diastolic |__|__|__| **DBP1**	2nd Pulse rate |__|__|__| **PRA2**
3rd Systolic BP |__|__|__| **SBP3**	3rd Diastolic |__|__|__| **DBP1**	3rd Pulse rate |__|__|__| **PRA3**

Mean blood pressure readings and blood pressure percentile

Mean Systolic	|__|__|__| **MSBP1**	Systolic percentile	|__|__|th **SCENTL1**
Mean Diastolic	|__|__|__| **MDBP1**	Diastolic percentile	|__|__|th **DCENTL1**

Classification of Blood pressure (according the BPS Classification code)

Classification of BP (answer **1**=Normal (**<90th BP percentile**), **2** = Prehypertension (**90th to <95th BP percentile**), **3**= Stage 1 hypertension (**95th percentile to the 99th percentile plus 5 mmHg**) and **4** =Stage 2 hypertension (> **99th percentile plus 5 mmHg**)

|___| **CLAS1**

NOTE *If answer is* **3, invite the child for day two for further BP measurement in two weeks' time.**
If answer is **4, invite the child for day two for further BP measurement in one weeks' time.**

Is the child eligible for second day BP measurement? (Answer 1=yes, 2=no) |___| **APOI2**

Date of second day appointment |__|__|/|__|__|/| 2 | 0 | 1 |__| **DATEAP2**

Investigator's ....

Name Signature

DAY TWO

Date of visit (dd/mm/201y) |__|__|/|__|__|/| 2 | 0 |1 |__| **DATEVB2**

First blood pressure reading

Take 3 reading, waiting 5 minutes between each reading

1st Systolic BP |__|__|__| **SBP21** 1ST Diastolic |__|__|__| **DBP21** 1st Pulse rate |__|__|__| **PRA21**

2nd Systolic BP |__|__|__| **SBP22** 2nd Diastolic |__|__|__| **DBP22** 2nd Pulse rate |__|__|__| **PRA22**

3rd Systolic BP |__|__|__| **SBP23** 3rd Diastolic |__|__|__| **DBP23** 3rd Pulse rate |__|__|__| **PRA32**

Mean blood pressure readings and Blood pressure percentile

Mean Systolic |__|__|__| **MSBP21** Systolic percentile |__|__|th **SCENTL21**

Mean Diastolic |__|__|__| **MDBP22** Diastolic percentile |__|__|th **DCENTL22**

Classification of Blood pressure (according the BPS Classification code)

Classification of BP (answer **1**=Normal (**<90th BP percentile**), **2** = Prehypertension (**90th to <95th BP percentile), 3**= Stage 1 hypertension (**95th percentile to the 99th percentile plus 5 mmHg**) and **4** =Stage 2 hypertension (> **99th percentile plus 5 mmHg**) |___| **CLAS2**

NOTE *If answer is* **3, invite the child for day two for further BP measurement in two weeks' time.**
If answer is **4, invite the child for day two for further BP measurement in one weeks' time.**

Is this child eligible for third day BP measurement? (Answer 1=yes, 2=no) |___| **APOI3**

Date of third day appointment |__|__|/|__|__|/| 2 | 0 | 1 |__| **DATEAP3**

Investigator's ....
Name Signature

DAY THREE

Date of Visit (dd/mm/201y) |__|__|/|__|__|/| 2 | 0 |1 |__| **DATEVB3**

First blood pressure and pulse rate reading

Take 3 reading, waiting 5 minutes between each reading

1st Systolic BP |__|__|__| **SBP31** 1ST Diastolic |__|__|__| **DBP31** 1st Pulse rate |__|__|__| **PRA31**

2nd Systolic BP |__|__|__| **SBP32** 2nd Diastolic |__|__|__| **DBP32** 2nd Pulse rate |__|__|__| **PRA32**

3rd Systolic BP |__|__|__| **SBP33** 3rd Diastolic |__|__|__| **DBP33** 3rd Pulse rate |__|__|__| **PRA33**

Mean blood pressure readings and Blood pressure percentile

Mean Systolic |__|__|__| **MSBP3** Systolic percentile |__|__|th **SCENTL3**

Mean Diastolic |__|__|__| **MDBP3** Diastolic percentile |__|__|th **DCENTL3**

If the mean systolic BP and/or diastolic BP is > 95th centile on day three, then the child has hypertension and BP should be staged

Classification of Blood pressure (according the BPS Classification code)

Classification of BP (answer **1**=Normal (**<90th BP percentile**), **2** = Prehypertension (**90th to <95th BP percentile), 3**= Stage 1 hypertension (**95th percentile to the 99th percentile plus 5 mmHg**) and **4** =Stage 2 hypertension (**>99th percentile plus 5 mmHg**) |___| **CLAS3**

NOTE
If answer is **3** or **4, investigate and refer for further management within one week.**

Investigator's ....
Name Signature

Appendix 10: Research paper 5: Effect of birth weight, exclusive breastfeeding and growth in infancy on fat mass and fat free mass indices in early adolescence

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
www.lshtm.ac.uk

Registry
T: +44(0)20 7299 4646
F: +44(0)20 7299 4656
E: registry@lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

RESEARCH PAPER COVER SHEET

PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	Abubaker Swaib Lule
Principal Supervisor	Emily Webb
Thesis Title	Investigating the impact of early-life, life-course and genetic factors on blood pressure among young Africans

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

**If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.*

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Current Developments in Nutrition
Please list the paper's authors in the intended authorship order:	Jonathan Nsamba, Swaib A. Lule, Benigna Namara, Christopher Zziwa, Helen Akurut, Lawrence Lubyayi, Florence Akello, Josephine Tumusiime, Alison M. Elliott, Emily L. Webb
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceptualised and designed the study, drafted the study documents (including protocol, standard operating procedures, questionnaires, consent and assent information sheets and forms), conducted the study (including data collection, data

	management and cleaning), performed statistical analysis, interpreted the findings and drafted, revised and submitted the final manuscript

Student Signature: [redacted] **Date:** 2/10/2018

Supervisor Signature: [redacted] **Date:** 2/10/2018

Effect of birth weight, exclusive breastfeeding and growth in infancy on fat mass and fat free mass indices in early adolescence[3]

*Jonathan Nsamba[1], *Swaib A. Lule[2, 3,] Benigna Namara[3], Christopher Zziwa[3], Helen Akurut[3], Lawrence Lubyayi[3], Florence Akello[3], Josephine Tumusiime[3], Alison M. Elliott[3,4], Emily L. Webb[2,5]

Author affiliations

[1]Department of Population Health, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

[2] Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

[3] Endemic, Neglected, Emerging and Re-emerging Infections, MRC/UVRI & LSHTM, Uganda Research Unit, P.O. Box 49, Entebbe, Uganda

[4] Department of Clinical Research, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

[5]Medical Research Council Tropical Epidemiology Group, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

Corresponding author: Jonathan Nsamba. Email: jonahnsamba@ymail.com

*These authors contributed equally

Authors' last names: Nsamba, Lule, Namara, Zziwa, Akurut, Lubyayi, Akello, Tumusiime, Elliott, Webb

Word Count:2677

Number of tables: 2

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[3]Supplementary tables 1, 2 and 3 are available in the supplementary data

Running title: Birth weight, breastfeeding, growth and body composition

Abbreviations used: BMI, Body Mass Index; CI, Confidence Interval; EMaBs, Entebbe Mother and Baby Study; FM, Fat Mass; FMI, Fat Mass Index; FFM, Fat Free Mass; FFMI, Fat Free Mass Index; NCDs, Non-Communicable Diseases; SD, Standard Deviation

Funding: The Entebbe Mother and Baby Study was supported by the Wellcome Trust through senior fellowship grants held by Prof. Elliott (grant numbers 064693, 079110, 95778) with supplementary funding from the UK Medical Research Council and UK Department for International Development (DfID) under the MRC/DfID concordat.

All authors declare no conflict of interest.

ABSTRACT

Background: There is limited data from Africa on the effect of pre- and post-natal growth and infant feeding on later body composition.

Objective: To investigate the effect of birth weight, exclusive breastfeeding and infant growth on adolescent body composition, using data from a Ugandan birth cohort.

Methods: Data was collected prenatally from pregnant women and prospectively from their resulting live offspring. Data on body composition parameters (fat mass index [FMI]and fat free mass index [FFMI]) was collected from 10- and 11-year olds in this cohort. Linear regression analysis was used to assess the effect of birth weight, exclusive breastfeeding and infant growth on FMI and FFMI, adjusting for confounders.

Results: 177 adolescents with a median age of 10.1 years were included in this analysis, with mean FMI 2.9 kg/m^2 (standard deviation (SD) 1.2), mean FFMI 12.8 kg/m^2 (SD 1.4) and mean birth weight 3.2 kg (SD 0.5). Ninety (50.9%) were male and 110 (63.2%) were exclusively breastfeeding at six weeks of age. In multivariable analysis, birth weight was associated with FMI (regression coefficient β= 0.66 per kg increase in birth weight, 95% confidence interval (CI) (0.04, 1.29), P=0.02), while exclusive breastfeeding (β= -0.43, 95% CI(-1.06, 0.19), P=0.12), growth 0-6 months (β= 0.24 95% CI(-0.43, 0.92), P=0.48) and growth 6-12 months (β= 0.61, 95% CI(-0.23, 1.46), P=0.11) were not associated with FMI among adolescents. Birth weight (β= 0.91, 95% CI (0.17, 1.65), P=0.01) was also associated with FFMI. Exclusive breastfeeding (β= 0.17, 95% CI (-0.60, 0.94), P=0.62), growth 0-6 months (β= 0.56, 95% CI (-0.20, 1.33), P= 0.10), and growth 6-12 months (β= -0.02, 95% CI (-1.02, 0.99), P=0.97) were not associated with FFMI.

Conclusions: Birth weight predicted body composition parameters in Ugandan early adolescents. However, exclusive breastfeeding at six weeks of age and growth in infancy were not associated with body composition at adolescence

Keywords: Birth weight, exclusive breastfeeding, infant growth, fat mass, fat free mass, adolescents, Uganda

Subject: Nutrition and Disease

Introduction

Previously neglected due to high burdens of infectious disease morbidity, attention paid to NCDs in Africa has recently increased. Studies suggest that high blood pressure (BP) (1,2) and other cardiovascular diseases (CVDs) (3) have escalated on the African continent over recent decades, disproportionately affecting populations at younger ages than in more affluent countries (4). The rising burden of non-communicable diseases (NCDs) in low and middle income countries is of public health and economic significance (5) given the fragile health care systems and associated cost implications. In Africa, deaths due to NCDs are rising faster than anywhere else in the world (4). An understanding of the pathways for development of NCDs in this setting is essential for informing interventions for prevention of NCDs.

Body composition, specifically increased adiposity, is associated with risk of NCDs later in life (6) and early-life factors, such as pre- and post-natal growth and infant feeding, have been reported to program and alter body composition (7). Sub-optimal nutrition in the fetal or infant periods triggers cellular and epigenetic changes that may affect later body composition (8). Rapid growth especially in infancy may result in metabolic changes which can manifest as increased adiposity and result in later NCDs (9,10). Thus, body composition change represents a mechanism through which early-life exposures may influence susceptibility to NCDs in adulthood.

Evidence, predominantly from high-income countries, has shown that compared to normal birth weight infants, both low and high birth weight infants may bear an increased risk of adulthood obesity (11). Rapid weight gain and lack of exclusive breastfeeding in infancy have been associated with increased adiposity in adulthood (12). Exclusive breastfeeding has also been reported to be associated with a reduction in fat mass (FM; a proxy for adiposity (13,14). However, results are inconsistent, with some studies finding no evidence for association between birth weight (low or high) and FM (7,11,15,16) in late adolescence or adulthood, or for an impact of these early-life factors on risk of NCDs later in life (17,18). Results (19) also suggest mixed evidence for an association between birth weight and fat free mass (FFM; a proxy for lean muscle mass (20)) in late adolescence or adulthood.

Few studies from Africa have investigated the relationship between birth weight, exclusive breastfeeding and growth in infancy, and body composition later in life, with tools for measuring body composition not widely available. Studies from South Africa (21) and

Cameroon (22) found that birth weight and linear growth were positively associated with both FM and FFM. However, the impact of early-life factors on later body composition remains under studied among populations from Africa.

Methods

The current study used prospectively collected data from the Entebbe Mother and Baby Study (EMaBS) birth cohort. The EMaBS started life as a randomised controlled trial of anthelminthic treatment interventions. It was conducted in Wakiso district, on the northern shores of Lake Victoria in Uganda. Between 2003 and 2005, pregnant women attending antenatal care at Entebbe Hospital and residing in Entebbe Municipality or Katabi sub-county were enrolled into a double-blind randomised placebo-controlled trial designed to evaluate the effect of deworming treatment in pregnancy and childhood on response to childhood vaccines and infections. A detailed description of the trial design has been given elsewhere (23).

We analysed prospectively collected data from the EMaBS, to investigate if birth weight, exclusive breastfeeding and growth in infancy were associated with body composition (fat mass index [FMI] and fat free mass indices [FFMI]) in early adolescence. Birth weight was measured and recorded to the nearest 0.1 kg for infants delivered in Entebbe hospital using weight scales (Fazzini SRL, Vimodrone, Italy), and captured as recorded on child health cards for infants delivered elsewhere. Further details have been reported previously (24).Weight was measured at six months and then annually starting at one year of age using weighing scales (Seca GmbH & Co. KG, Hamburg, Germany). Height was measured at six months and then annually to the nearest 0.1cm using stadiometers (Seca213 GmbH & Co. KG, Hamburg, Germany). Information on feeding practices was obtained at six weeks of age. The trial was completed in 2011 when all children had turned five years of age. After the trial completion, the offspring continued under follow up, being seen at annual routine visits and when sick.

Between 20th May 2014 and 16th June 2016, 10- and 11-year olds in the EMaBS attending the study clinic for their annual visit were enrolled into the EMaBS blood pressure study (BPS). The primary aim of the EMaBS BPS was to investigate whether birth weight and pre- and peri-natal exposures, and genetic polymorphisms are important in programming BP in Ugandan adolescents; further details are described elsewhere (25). From 21st January 2015

to 23rd December 2015, additional data on body composition (FM and FFM) was collected from EMaBS participants enrolled into the BPS; outside this period the body composition analyser machine was not available. Briefly, adolescents stood barefoot on the posterior electrode base while holding strongly the two anterior electrodes handles of the segmental body composition analyser machine (TANITA BC-418, TANITA Corporation, Tokyo Japan). To avoid ambiguities from using body composition percentages (26,27), height normalized indices (FMI in kg/m^2 and FFMI in kg/m^2) were computed and used for analysis. FMI is considered as a measure of adiposity and FFMI as a measure of lean muscle mass.

Statistical methods

Study exposures were birth weight, breastfeeding status at six weeks, early infant growth (0-6 months) and late infant growth (6-12 months), while the study outcomes were FMI and FFMI at 10 or 11 years of age. Birth weight was considered for analysis as both a continuous variable and as a categorical variable (low birth weight <2.5kg, normal weight 2.5-3.5kg and high birth weight >3.5kg). The 2006 World Health Organisation(WHO) growth standards (28) were used to compute weight for age standardised Z-scores at birth, and at six and 12 months of age. For each participant, growth for the periods 0-6 months and 6-12 months was calculated as the change in Z-score during that period. Growth in each time period (0-6 months, 6-12 months) was categorised as either increased or normal growth using the cut-off of a 0.67 increase in z-score (10,29)

Characteristics of study participants were compared with those of cohort members who did not participate using t-test and chi-squared tests. Descriptive statistics were calculated as frequencies, means and standard deviations. Spearman's correlation was used to assess correlations of body composition indices with each other and with birth weight. Linear regression models were fitted separately for FMI and FFMI, adjusting for confounders. Potential confounders considered were maternal age, body Mass Index (BMI), education, area of residence and HIV status; household socio-economic status at enrolment; and offspring's place of delivery, sex, age at body composition analysis, family history of hypertension, type of school attended, days/week animal-proteins were eaten, days/week fruits were eaten, days/week vegetables were eaten, days/week starchy food were eaten, days/week sugared drinks were taken. Factors associated with the outcome, or with the

exposure of interest were added to the model concurrently and likelihood ratio tests were used to assess adjusted associations between each variable and the outcome.

Current BMI, which can be partitioned into FMI plus FFMI, was considered to be on the causal pathway between birth weight and FMI or FFMI, thus was not considered as a potential confounder for inclusion in regression models. Assumptions underlying the linear regression model analysis (linear relationship between the dependent and predictor variables, homoscedasticity, normally distributed residuals) were investigated using a combination of scatter plots, plots of residuals against fitted values, and normal probability plots. The possibility of multicollinearity due to inclusion of correlated predictor variables was assessed using the change in standard error method.

For each of the main exposures, factors associated with that exposure or with the outcome at a 5% level of significance were included in the final model for that exposure. Three a priori confounders, household socio-economic status, age and sex were included in the final model regardless of whether associated with the exposure or outcome or not. The test for trend was used to investigate the shape of the relationship between birth weight and the outcomes. Likelihood ratio test p-values were calculated. STATA version 14.2 (College Station, Texas, USA) was used for data analysis. Interaction terms were fitted to assess whether birth weight might modify the effect of breastfeeding or increased growth on the outcomes (FMI or FFMI).

The EMaBS was granted ethical approval by the Uganda Virus Research Institute Science and Ethics Committee, the Ethics committee of the London School of Hygiene and Tropical Medicine, and the Uganda National Council of Science and Technology.

Results

Of the 2,345 live born EMaBS offspring, 1,119 (47.7%) enrolled into the BP study (25) at 10 or 11 years of age and 177 (7.6%) had data on body composition taken and were included in the analysis. Of the 177 participants included, 80 (50.9%) were male; 175 (98.9%) were singleton births; and 161 (91.0%) were not exposed to maternal HIV in pregnancy, **see table 1**. Regarding the key exposures, the mean birth weight was 3.2 kg (standard deviation (SD) 0.5); 13 (9.4%) had low birth weight, 92 (66.2%) normal birth weight and 34 (24.5%) high birth weight with 38 participants of unknown birth weight. One hundred and ten (63.2%)

were exclusively breastfed at six weeks of age; with three participants missing data on this exposure. One hundred and eight (61%) and 123 (69%) participants had information on growth between 0 and 6 months, and between 6 and 12 months, respectively (the remaining were missing anthropometry for at least one of the time points and thus the change in z-score could not be calculated); 35 (32.4%) had increased growth in the first 6 months of life and 15 (12.2%) had increased growth between 6 and 12 months of age.

Adolescents who had body composition measured were similar to original EMaBS cohort members who did not participate for most characteristics, except participants were more likely to be born to separated/divorced/widowed mothers (P-value=0.037) and were less likely to be born to mothers with hookworm infections in pregnancy (P-value=0.036).

At participation, offspring had a median age of 10.1 years (IQR: 10.0 to 10.7), mean BMI 15.8 kg/m^2 (SD 1.9), mean FMI 2.9 kg/m^2 (SD 1.2) and mean FFMI 12.8 kg/m^2 (SD 1.4). Among males, the mean FMI was 2.7 kg/m^2 (SD 1.3) and mean FFMI was 13.3 kg/m^2 (SD 1.1), while in females the mean FMI was 3.1 kg/m^2 (SD 0.9) and mean FFMI was 12.4 kg/m^2 (SD 1.5), **see Figure 1**. Birth weight was positively correlated with both FMI (r=0.35, p-value<0.001) and FFMI (r=0.34, p-value<0.001). There was strong correlation between FMI and FFMI with r=0.517, p-value <0.001.

The relationships between the main exposures, and FMI and FFMI are shown in **table 2**. Unadjusted estimates show that FMI increased by 0.73 kg/m^2 per unit kilogram increase in birth weight, 95% confidence interval (CI):0.33-1.13. When birth weight was treated as a categorical variable, it showed a dose-response relationship with FMI (P-trend=0.007). Further investigation of this dose-response relationship showed no departure from linearity (P=0.92). Exclusive breastfeeding at six weeks (β= -0.19, 95% CI: -0.55, 0.17), increased growth between birth and 6 months of age (β= 0.15, 95% CI: -0.42, 0.71) and increased growth between 6 and 12 months (β= 0.62, 95% CI: -0.10, 1.33) were not associated with FMI in unadjusted analysis. In multivariable analysis birth weight (β= 0.66, 95% CI: 0.04, 1.29) remained associated with FMI; exclusive breastfeeding at six weeks (β= -0.43, 95% CI: -1.06, 0.19), increased growth between birth and 6 months of age (β= 0.24 95% CI: -0.43, 0.92) and increased growth between 6 and 12 months (β= 0.61, 95% CI: -0.23, 1.46) were not associated with FMI.

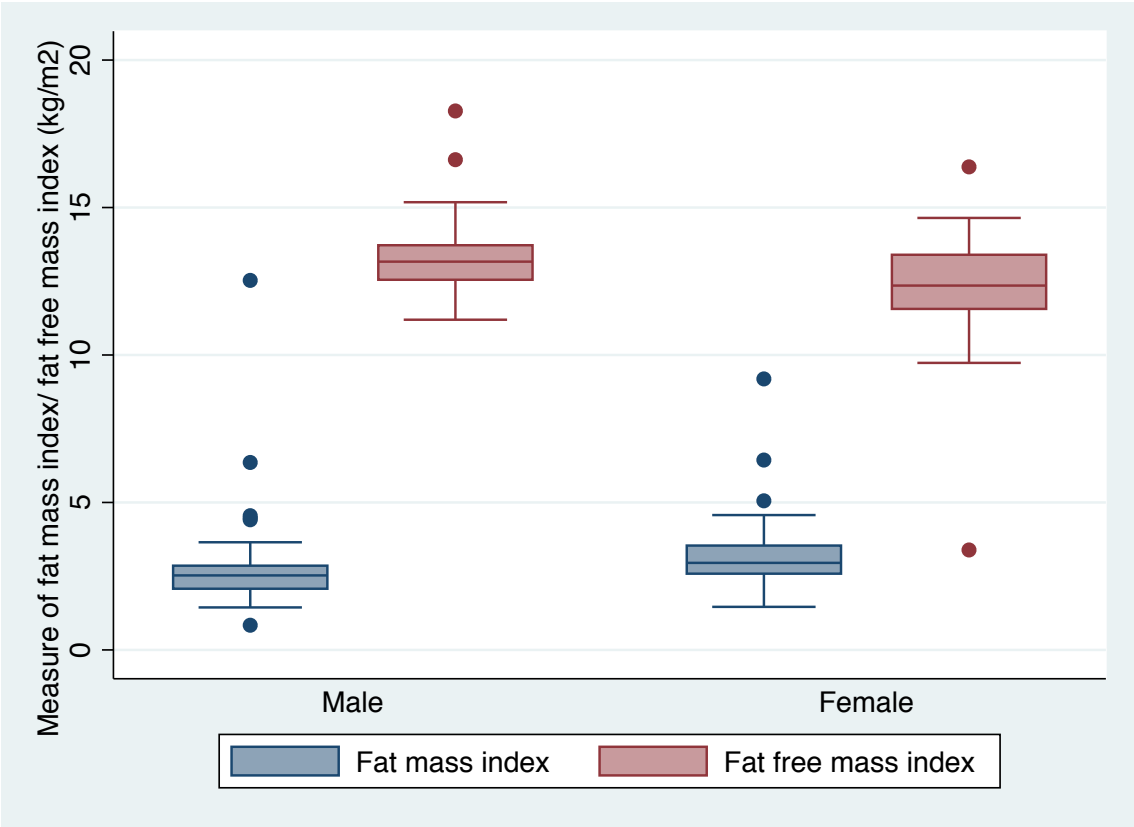

Figure 1: Distribution of fat mass index and fat free mass index by sex

Birth weight was positively associated with FFMI in unadjusted analysis (β= 0.68, 95% CI: 0.21, 1.16), while exclusive breastfeeding at six weeks (β= 0.14 95% CI: -0.30, 0.57), increased growth between birth and 6 months of age (β= 0.36, 95% CI; -0.29, 1.00) and increased growth between 6 and 12 months (β= -0.51, 95% CI: -1.33, 0.32) were not associated with FFMI. When birth weight was analysed as a categorical variable, findings were consistent with a linear relationship with FFMI (P-trend=0.009, p-value for departure from trend 0.93). In multivariable analysis, birth weight (β= 0.91, 95% CI: 0.17, 1.65) remained associated with FFMI; there remained no evidence of association for the other exposures. There was no evidence that the effect of breastfeeding or growth rate on FMI or FFMI differed by sex or birth weight: for example, for FMI, p-values were 0.97, 0.47 and 0.60 for interaction between birth weight and breastfeeding, growth 0-6 months and growth 6-12 months, respectively. The corresponding interaction p-values for FFMI were 0.12, 0.13 and 0.16, respectively. For all analyses, assessment of the assumptions underlying the linear regression analysis indicated that these were met, and there was no suggestion of multicollinearity.

Table 1 Participant characteristics (N=177)

Characteristics	Frequency/ Mean (sd)	Percentage
Maternal at enrolment		
Age, years	24.7 (6.1)	
Household economic status (1 lowest, 6 highest) (n=176)	3.8 (1.1)	
Body mass index (kg/m^2)	24.5 (3.3)	
Area of residence (n=176)		
Urban	114	64.8
Rural	62	35.2
Education		
None	4	2.3
Primary	77	43.5
Secondary	76	42.9
Tertiary	20	11.3
HIV status		
Negative	161	91.0
Positive	16	9.0
Offspring		
Age, years	10.4(0.5)	
Birth weight, kg (n=139)	3.2 (0.5)	
Fat mass index	2.9 (1.2)	
Fat free mass index	12.8 (1.4)	
Sex		
Male	90	50.9
Female	87	49.2
Exclusively breastfed at 6 weeks (n=174)		
No	64	36.9
Yes	110	63.2
Place of birth		
Entebbe Hospital	127	71.8
Home	20	11.3
Other places	30	17.0
HIV status		
Unexposed	161	91.0
Exposed not infected	14	7.9
Infected	2	1.1
Public hair development (n=174)		
Pre-pubertal	128	73.6
Pubertal	46	26.4
Breast development (girls only) (n=83)		
Pre-pubertal	66	79.5
Pubertal	17	20.5
Days fruit eaten/week (n=174)		
None	13	7.5
1-3	113	64.9
4-7	48	27.6
Days vegetables eaten/week (n=176)		
None	15	8.5
1-3	101	57.4
4-7	60	34.1
Days animal-protein eaten/week (n=176)		
None	14	8.0
1-3	133	75.6
4-7	29	16.5
Days starchy food eaten/week		
1-3	4	2.3
4-7	173	97.7
Days sugared drinks taken/week		
None	63	36.2
1-3	82	46.3
4-7	31	17.5
Type of school (n=176)		
Boarding	27	15.3
Day	149	84.7

Percentages may be ± 100 due rounding.
SD; standard deviation.
Missing data: area of residence 1; birth weight 38; pubic hair development 3; breast development 4; days fruit eaten/week 3; days vegetables eaten/week 1; days proteins eaten/week 1; type of school 1.

Table 2 **Unadjusted and adjusted associations between birth weight, exclusive breastfeeding and growth in infancy, and body composition outcomes (N=177)**

Exposures	Unadjusted		Adjusted	
	β (95 % CI)	p-value	β (95 % CI)	p-value*
Fat mass index				
Birth weight (continuous) (n=139)	0.73 (0.33, 1.13)	<0.001	0.66 (0.04, 1.29)	0.019
Birth weight (categorical)				
<2.5 kg (n=13)	Reference		Reference	
2.5 to 3.5 (n=92)	0.54 (-0.18, 1.26)		0.87 (-0.06, 1.80)	
> 3.5 kg (n=34)	1.03 (0.24, 1.82)	0.007 [trend]	1.09 (-0.04, 2.23)	0.051 [trend]
Exclusively breastfed at 6 weeks				
No (n=64)	Reference		Reference	
Yes (n=110)	-0.19 (-0.55, 0.17)	0.538	-0.43 (-1.06, 0.19)	0.122
Growth between 0 to 6 months				
Normal (n=73)	Reference		Reference	
Increased (n=35)	0.15 (-0.42, 0.71)	0.600	0.24 (-0.43, 0.92)	0.480
Growth between 6 to 12 months				
Normal (n=108)	Reference		Reference	
Increased (n=15)	0.62 (-0.10, 1.33)	0.089	0.61 (-0.23, 1.46)	0.107
Fat free mass index				
Birth weight (continuous) (n=139)	0.68 (0.21, 1.16)	0.005	0.91 (0.17, 1.65)	0.007
Birth weight (categorical)				
> 2.5 kg (n=13)	Reference		Reference	
2.5 to 3.5 (n=92)	0.61 (-0.24, 1.45)		1.11 (0.01, 2.21)	
> 3.5 kg (n=34)	1.16 (0.23, 2.09)	0.009 [trend]	1.53 (0.19, 2.87)	0.020 [trend]
Exclusively breastfed at 6 weeks				
No (n=64)	Reference		Reference	
Yes (n=110)	0.14 (-0.30, 0.57)	0.538	0.17 (-0.60, 0.94)	0.619
Growth between 0 to 6 months				
Normal (n=73)	Reference		reference	
Increased (n=35)	0.36 (-0.29, 1.00)	0.272	0.56 (-0.20, 1.33)	0.100
Growth between 6 to 12 months				
Normal (n=108)	Reference		Reference	
Increased (n=15)	-0.51 (-1.33, 0.32)	0.224	-0.02 (-1.02, 0.99)	0.971

In multivariable analysis, all factors shown in the table were added to the model together. Adjusted associations were adjusted for maternal characteristics at enrolment (household socio-economic status, age, body mass index, HIV status) and adolescents' characteristics (place of delivery, age, sex, days animal-protein eaten/week, days fruits eaten/week)

* likelihood ratio test p-value

Discussion

We hypothesised that birth weight, exclusive breastfeeding and rate of growth were each associated with body composition indices among Ugandan adolescents aged 10-11 years. This study showed that birth weight was associated with both adolescent FMI and adolescent FFMI but there was no association between exclusive breastfeeding in the first six weeks or growth rate in infancy and FMI or FFMI among early adolescents.

Our findings of a positive association between birth weight and both FMI and FFMI are consistent with results from across-sectional study among 557 Cameroonian children aged 5-12 years (22) and from a birth cohort study among South Africans, with body composition assessed at ages10 and 22 years (21,30).

We did not find evidence for an effect of exclusive breastfeeding in the first six weeks on FMI or FFMI. This was contrary to results reported in a meta-analysis (31) that showed that on average, each additional month of exclusive breastfeeding reduced adiposity by 4%.The lack of association between exclusive breastfeeding in the first six weeks with adiposity or lean muscle mass development in this study supports results among 18 year old Brazilians enrolled in a population based birth cohort (32). In our study, only 63% of mothers reported exclusive breastfeeding at six weeks but nearly all mothers [172 (97.2%)] were giving some breast milk and only 2 (1.1%) had weaned, thus a differential effect of breast milk may be hard to detect in this population. The relationship between exclusive breastfeeding in the first six months of life and adolescents' body composition was not examined because data on feeding status at six months was not collected.

There was no association between increased rate of growth in the first six months of life or from 6 to 12 months and FMI or FFMI. These findings do not support earlier studies predominantly from European counties reviewed in (18,33) and results from a later study among 909 Dutch term infants (33) which reported positive associations between growth rate and body composition. Our study was likely underpowered to detect true associations: of the 177 adolescents for whom body composition data were available, data on growth were only available for around two thirds, thus reducing the sample size for this analysis. Among participants in the larger EMaBS BPS (1,119 participants, of which the 177 participants with body composition data were a subset), growth in the first two years of life was positively associated with BP in early adolescence (25).

Many studies have used body mass index (BMI) as a surrogate outcome measure for body adiposity. However, evidence to date shows that BMI creates ambiguities since it cannot specifically differentiate between FM and FFM (34). We therefore used direct measurement of body composition and the height normalised indices for FM and FFM which are reported to be more precise measures of adiposity and lean muscle, respectively (26). The strong correlation between FMI and FFMI suggests that, for the Uganda adolescents participating in our study, FMI and FFMI both increase proportionally with an increase in BMI. This is reflected by the fact that birth weight was positively associated with both increased adiposity and increased lean muscle mass in early adolescence.

We used a bio-electrical impendence body composition analyser machine to measure segmental body composition among the study adolescents. Bio-electrical impendence has been reported to have good correlation with other methods such as dual energy absorptiometry (35) and, importantly in this setting, provided a relatively inexpensive field method of body composition analysis.

To our knowledge, this was the first study in East Africa to investigate the impact of early-life factors on the body composition parameters FMI and FFMI. Strengths of the study are its cohort design and the robust methods used for measuring body composition parameters. Data on the exposures of interest and potential confounders were collected prospectively, minimizing recall and reporter bias. Exposures and confounders were determined before the BP study was conceptualized and designed. However, the possibility of residual confounding due to unmeasured variables cannot be ruled out. Some exposure information such as exclusive breastfeeding at six weeks was not available for all of the adolescents. In this study we were unable to differentiate the effects of low birth weight due to growth restriction in utero from effects due to pre-term birth because accurate data on gestational age was not available in this population.

Whereas we have investigated the effect of two postnatal factors (rate of growth and exclusive breastfeeding) on later disease risk, further studies should investigate the effect of other factors such as current diet, age at menarche, sleep patterns/ duration and the effect of an obesogenic environment on body composition. In conclusion, exclusive breastfeeding, and infant growth were not associated with body composition among early adolescents from a tropical setting. However, birth weight is a good predictor of both adiposity and lean muscle mass later in life in this setting.

Acknowledgments and statement of authors' contributions to manuscript

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A.M.E., E.L.W. and S.A.L. conceptualised and designed the study; B.N., C.Z., J.T., A.F., L.L., and S.A.L. conducted the study; J.N., S.A..L and E.L.W. performed statistical analysis and interpreted findings; J.N., S.A.L. and E.L.W. wrote the manuscript; and E.L.W had primary responsibility for final content. All authors read and approved the final manuscript.

Supplementary tables

Table S1 Unadjusted associations of maternal, childhood and adolescence characteristics with birth weight and exclusive breastfeeding

Characteristics	Birth weight		Exclusive breast feeding	
Maternal at enrolment	**β (95 % CI)**	**p-value**	**β (95 % CI)**	**p-value**
Age	**0.02 (0.01, 0.03)**	**0.003**	-0.01 (-0.02, 0.00)	0.059
Household economic status	0.06 (-0.01, 0.13)	0.086	**-0.08 (-0.14, -0.02)**	**0.014**
Body mass index	**0.03 (0.01, 0.06)**	**0.007**	-0.00 (-0.03, 0.02)	0.657
Area of residence				
Urban	Reference		Reference	
real	-0.12 (-0.20, 0.17)	0.869	-0.06 (-0.21, 0.10)	0.473
Education				
None	-0.43 (-1.44, 0.58)		0.21 (-0.28, 0.70)	
Primary	Reference		Reference	
Senior	0.00 (-0.18, 0.19)		0.15 (-0.01, 0.30)	
Tertiary	0.19 (-0.07, 0.45)	0.377	0.21 (-0.03, 0.45)	0.154
HIV status				
Negative	Reference		**Reference**	
Positive	0.01 (-0.29, 0.32)	0.941	**-0.33 (-0.58, -0.07)**	**0.012**
Childhood				
Birth weight	-		0.03 (-0.13, 0.19)	0.716
Sex				
Male	Reference		Reference	
Female	-0.02 (-0.19, 0.15)	0.825	0.08 (-0.7, 0.22)	0.307
Exclusively breastfed by 6 weeks				
No	Reference			
Yes	0.03 (-0.15, 0.21)	0.716	-	
Place of birth				
Entebbe Hospital	**Reference**		**Reference**	
Home	**-0.68 (-1.18, -0.18)**		**-0.11 (-0.34, 0.12)**	
Other places	**-0.07 (-0.38, 0.24)**	**0.029**	**-0.25 (-0.45, -0.06)**	**0.030**
HIV status				
Unexposed	Reference		**Reference**	
Exposed not infected	0.04 (-0.29, 0.374)		**-0.30 (-0.57, -0.04)**	
Infected	-0.15 (-0.87, 0.57)	0.885	**-0.66 (-1.60, 0.28)**	**0.033**
Adolescence				
Age	**0.23 (0.07, 0.39)**	**0.005**	**-0.14 (-0.27, -0.01)**	**0.040**
Body mass index	**0.10 (0.06, 0.14)**	**<0.001**	-0.01 (-0.05, 0.03)	0.551
Public hair development				
Pre-pubertal	Reference		Reference	
Pubertal	-0.08 (-0.28, 0.12)	0.406	-0.07 (-0.24, 0.09)	0.386
Breast development (girls only)				
Pre-pubertal	Reference		Reference	
Pubertal	0.00 (-0.28, 0.29)	0.980	-0.19 (-0.46, 0.07)	0.151
Days a fruit is eaten/week				
None	Reference		Reference	
1-3	0.04 (-0.28, 0.36)		-0.14 (-0.42, 0.14)	
4-7	0.26 (-0.08, 0.60)	0.066	-0.13 (-0.43, 0.17)	0.617
Days vegetables is eaten/week				
None	Reference		Reference	
1-3	-0.05 (-0.37, 0.28)		-0.05 (-0.32, 0.21)	
4-7	0.01 (-0.33, 0.35)	0.807	-0.01 (-0.28, 0.27)	0.826
Days animal-protein eaten/week				
None	**Reference**		**Reference**	
1-3	**-0.10 (-0.41, 0.21)**		**-0.03 (-0.30, 0.24)**	
4-7	**0.21 (-0.15, 0.57)**	**0.034**	**0.08 (-0.23, 0.39)**	**0.521**
Days starchy food eaten/week				
1-3	Reference		Reference	
4-7	0.10 (-0.48, 0.69)	0.730	-0.12 (-0.60, 0.36)	0.623
Days sugared drinks taken/week				
None	Reference		Reference	

1-3	-0.04 (-0.23, 0.15)		0.01 (-0.15, 0.17)	
4-7	-0.07 (-0.31, 0.18)	0.849	0.18 (-0.03, 0.39)	0.196
Type of school				
boarding	Reference		Reference	
Day	-0.20 (-0.43, 0.04)	0.098	0.00 (-0.20, 0.20)	0.996
Family history of hypertension				
No	Reference		Reference	
Yes	-0.10 (-0.38, 0.18)	0.479	-0.00 (-0.26, 0.25)	0.980

Percentages may be ± 100 due rounding.

Missing data: area of residence 1; birth weight 38; pubic hair development 1; breast development 4; days a fruit is eaten/week 3; days vegetables are eaten/week 1; days proteins are eaten/week 1; type of school 1.

Table S2 Unadjusted associations of maternal, childhood and adolescence characteristics with increased growth for given periods

Characteristics	0-6 months of age		6-12 months of age	
Maternal at enrolment	**β (95 % CI)**	**p-value**	**β (95 % CI)**	**p-value**
Age	0.00 (-0.01, 0.02)	0.731	0.00 (-0.01, 0.01)	0.532
Household economic status	0.01 (-0.07, 0.85)	0.827	0.03(-0.02, 0.08)	0.245
Body mass index	-0.00 (-0.03, 0.03)	0.972	**0.03 (0.01, 0.05)**	**0.001**
Area of residence				
Urban	Reference		Reference	
real	0.07 (-0.13, 0.27)	0.495	-0.01 (-0.13, 0.12)	0.906
Education				
None	-0.30 (-1.26, 0.65)		-0.17 (-0.64, 0.29)	
Primary	Reference		Reference	
Senior	0.03 (-0.17, 0.23)		-0.06 (-0.19, 0.06)	
Tertiary	0.07 (-0.20, 0.34)	0.862	-0.17 (-0.37, 0.02)	0.324
HIV status				
Negative	Reference		Reference	
Positive	-0.19 (-0.56, 0.17)	0.294	-0.13 (-0.37, 0.11)	0.277
Childhood				
Birth weight	**-0.31 (-0.48, -0.15)**	**<0.001**	-0.04 (-0.18, 0.09)	0.508
Sex				
Male	Reference		Reference	
Female	-0.05 (-0.23, 0.13)	0.549	-0.00 (-0.12, 0.12)	0.996
Exclusively breastfed by 6 weeks				
No	Reference		**Reference**	
Yes	0.07 (-0.13, 0.27)	0.483	**-0.14 (-0.27, -0.01)**	**0.033**
Place of birth				
Entebbe Hospital	Reference		Reference	
Home	-0.31 (-0.96, 0.35)		0.04 (-0.16, 0.25)	
Other places	0.32 (-0.02, 0.66)	0.112	-0.14 (-0.31, 0.04)	0.255
HIV status				
Unexposed	Reference		Reference	
Exposed not infected	-0.19 (-0.56, 0.17)		-0.13 (-0.37, 0.11)	
Infected	-	0.294	**-**	0.277
Adolescence				
Age	-0.10 (-0.27, 0.06)	0.229	**0.09 (-0.02, 0.20)**	**0.097**
Body mass index	0.02 (-0.93, 0.06)	0.447	0.01 (-0.02, 0.04)	0.446
Public hair development				
Pre-pubertal	Reference		**Reference**	
Pubertal	0.05 (-0.16, 0.25)	0.663	**-0.12 (-0.26, 0.02)**	**0.089**
Breast development (girls only)				
Pre-pubertal	Reference		Reference	
Pubertal	0.08 (-0.23, 0.40)	0.592	0.09 (-0.15, 0.33)	0.472
Days a fruit is eaten/week				
None	Reference		Reference	
1-3	-0.03 (-0.34, 0.28)		0.04 (-0.17, 0.26)	
4-7	-0.21 (-0.55, 0.14)	0.242	-0.06 (-0.30, 0.17)	0.299
Days vegetables is eaten/week				
None	Reference		Reference	
1-3	0.27 (-0.06, 0.60)		0.00 (-0.23, 0.23)	
4-7	0.17 (-0.17, 0.52)	0.227	0.01 (-0.22, 0.25)	0.976
Days animal-protein eaten/week				
None	Reference		Reference	
1-3	0.08 (-0.25, 0.40)		0.14 (-0.06, 0.34)	
4-7	0.36 (-0.05, 0.77)	0.115	-0.14 (-0.11, 0.40)	0.399
Days starchy food eaten/week				
1-3	Reference		Reference	
4-7	0.33 (-0.34, 1.00)	0.328	0.16 (-0.26, 0.51)	0.515
Days sugared drinks taken/week				
None	Reference		Reference	
1-3	-0.00 (-0.20, 0.20)		0.111 (-0.02, 0.24)	
4-7	0.19 (-0.07, 0.44)	0.258	-0.-3 (-0.20, 0.14)	0.114
Type of school				

boarding	Reference		Reference	
Day	0.07 (-0.16, 0.31)	0.536	-0.02 (-0.18, 0.13)	0.763
Family history of hypertension				
No	Reference		Reference	
Yes	0.19 (-0.09, 0.48)	0.179	0.06 (-0.14, 0.27)	

Percentages may be ± 100 due rounding.

Missing data: area of residence 1; birth weight 38; pubic hair development 1; breast development 4; days a fruit is eaten/week 3; days vegetables are eaten/week 1; days proteins are eaten/week 1; type of school 1.

Table S3 Unadjusted associations of maternal, childhood and adolescence characteristics with fat mass index and fat free mass index

Characteristics	Fat mass index		Fat free mass index	
Maternal at enrolment	**β (95 % CI)**	**p-value**	**β (95 % CI)**	**p-value**
Age	0.02 (-0.01, 0.05)	0.117	-0.00 (-0.04, 0.03)	0.810
Household economic status	0.12 (-0.03, 0.27)	0.123	0.08 (-0.10, 0.26)	0.369
Body mass index	**0.12 (0.07, 0.17)**	**<0.001**	0.06 (-0.00, 0.12)	0.056
Area of residence				
Urban	Reference		Reference	
real	0.05 (-0.32, 0.41)	0.794	-0.17 (-0.60, 0.26)	0.444
Education				
None	**-0.22 (-1.36, 0.92)**		-.026 (-1.65, 1.12)	
Primary	**Reference**		Reference	
Senior	**0.10 (-0.26, 46)**		0.02 (-0.41, 0.46)	
Tertiary	**1.01 (0.45, 1.57)**	**0.004**	0.75 (0.07, 1.43)	0.149
HIV status				
Negative	Reference		Reference	
Positive	-0.12 (-0.72, 0.48)	0.688	-0.03 (-0.74, 0.68)	0.925
Childhood				
Birth weight	**0.73 (0.33, 1.13)**	**<0.001**	**0.68 (0.21, 1.16)**	**0.005**
Sex				
Male	**Reference**		**Reference**	
Female	**0.44 (0.10, 0.77)**	**0.012**	**-0.87 (-1.26, -0.46)**	**<0.001**
Exclusively breastfed at 6 weeks				
No	Reference		Reference	
Yes	-0.19 (-0.55, 0.17)	0.299	0.14 (-0.30, 0.57)	0.538
Place of birth				
Entebbe Hospital	Reference		Reference	
Home	-0.50 (-1.05, 0.05)		-0.29 (-0.94, 0.37)	
Other	0.07 (-0.39, 0.53)	0.167	0.32 (-0.23, 0.87)	0.300
HIV status				
Unexposed	Reference		Reference	
Exposed not infected	-0.10 (-0.74, 0.55)		-0.09 (-0.86, 0.67)	
Infected	-0.31 (-1.94, 1.33)	0.897	0.38 (-1.56, 2.33)	0.878
Adolescence				
Age	0.18 (-0.13, 0.50)	0.256	0.23 (-0.14, 0.61)	0.224
Body mass index	**0.49 (0.44, 0.54)**	**<0.001**	**0.52 (0.44, 0.59)**	**<0.001**
Public hair development				
Pre-pubertal	Reference		Reference	
Pubertal	-0.01 (-0.30, 0.29)	0.963	0.30 (-0.14, 0.74)	0.177
Breast development (girls only)				
Pre-pubertal	Reference		**Reference**	
Pubertal	0.46 (-0.03, 0.95)	0.066	**1.27 (0.51, 2.02)**	**0.001**
Days a fruit eaten/week				
None	Reference		**Reference**	
1-3	0.34 (-0.33, 1.02)		**0.32 (-0.47, 1.10)**	
4-7	0.62 (-0.09, 1.34)	0.171	**0.92 (0.08, 1.76)**	**0.018**
Days vegetables eaten/week				
None	Reference		Reference	
1-3	0.53 (-0.10, 1.16)		0.36 (-0.40, 1.12)	
4-7	0.23 (-0.43, 0.89)	0.118	0.35 (-0.44, 1.14)	0.634
Days animal-protein eaten/week				
None	Reference		Reference	
1-3	0.23 (-0.42, 0.87)		-0.09 (-0.86, 0.68)	
4-7	0.46 (-0.29, 1.21)	0.451	-0.31 (-1.20, 0.58)	0.697
Days starchy food eaten/week				
1-3	Reference		Reference	
4-7	0.04 (-1.12, 1.21)	0.941	-0.61 (-1.99, 0.77)	0.384
Days sugared drinks taken/week				
None	Reference		Reference	
1-3	0.26 (-0.12, 0.65)		-0.13 (-0.58, 0.33)	
4-7	0.07 (-0.43, 0.57)	0.378	0.18 (-0.41, 0.78)	0.559
Type of school				

boarding	Reference		Reference	
Day	-0.72 (-1.19, -0.25)	0.003	0.06 (-0.51, 0.63)	0.832
Family history of hypertension				
No	Reference		Reference	
Yes	0.51 (-0.09, 1.11)	0.093	-0.01 (-0.73, 7.0)	0.970

Percentages may be ± 100 due rounding.

Missing data: area of residence 1; birth weight 38; pubic hair development 1; breast development 4; days a fruit is eaten/week 3; days vegetables are eaten/week 1; days proteins are eaten/week 1; type of school 1.

References

1. Noubiap JJ, Essouma M, Bigna JJ, Jingi AM, Aminde LN, Nansseu JR. Prevalence of elevated blood pressure in children and adolescents in Africa: a systematic review and meta-analysis. Lancet Public Health. 2017 Aug 1;2(8):e375–86.

2. Addo J, Smeeth L, Leon DA. Hypertension In Sub-Saharan Africa: A Systematic Review. Hypertension. 2007 Dec;50(6):1012–8.

3. Dalal S, Beunza JJ, Volmink J, Adebamowo C, Bajunirwe F, Njelekela M, et al. Non-communicable diseases in sub-Saharan Africa: what we know now. Int J Epidemiol. 2011 Aug 1;40(4):885–901.

4. Miranda JJ, Kinra S, Casas JP, Smith GD, Ebrahim S. Non-communicable diseases in low- and middle-income countries: context, determinants and health policy. Trop Med Int Health TM IH. 2008 Oct;13(10):1225–34.

5. Rath S, Tariq M, Mushoriwa F, Chigumete T, Morobi T, Srinivas S. Economics of Non-Communicable Diseases: Case Study of South Africa and India. Indian J Pharm Pract. 2015;8(3):91.

6. Must A, McKeown N. The Disease Burden Associated with Overweight and Obesity. South Dartmouth (MA): MDText.com, Inc; 2012.

7. Victora CG, Sibbritt D, Horta BL, Lima RC, ScD TC, Wells J. Weight gain in childhood and body composition at 18 years of age in Brazilian males. Acta Paediatr. 2007 Feb;96(2):296–300.

8. Bateson P, Barker D, Clutton-Brock T, Deb D, D'Udine B, Foley RA, et al. Developmental plasticity and human health. Nature. 2004 Jul 22;430(6998):419–21.

9. Stutte S, Gohlke B, Peiler A, Schreiner F, Born M, Bartmann P, et al. Impact of Early Nutrition on Body Composition in Children Aged 9.5 Years Born with Extremely Low Birth Weight. Nutrients. 2017 Feb 10;9(2):124.

10. Raaijmakers A, Jacobs L, Rayyan M, van Tienoven TP, Ortibus E, Levtchenko E, et al. Catch–up growth in the first two years of life in Extremely Low Birth Weight (ELBW) infants is associated with lower body fat in young adolescence. PLOS ONE. 2017 Mar 9;12(3):e0173349.

11. Barker M, Robinson S, Osmond C, Barker DJ. Birth weight and body fat distribution in adolescent girls. Arch Dis Child. 1997 Nov;77(5):381–3.

12. Ellis KJ. Body composition in infancy: impact on health later in life. Nestle Nutr Workshop Ser Paediatr Programme. 2010;65:213–20; discussion 221-224.

13. Yin J, Quinn S, Dwyer T, Ponsonby AL, Jones G. Maternal diet, breastfeeding and adolescent body composition: a 16-year prospective study. Eur J Clin Nutr. 2012;66(12):1329–1334.

14. Ramírez-Vélez R, Correa-Bautista JE, Sanders-Tordecilla A, Ojeda-Pardo ML, Cobo-Mejía EA, Castellanos-Vega R del P, et al. Percentage of Body Fat and Fat Mass Index as a Screening Tool for Metabolic Syndrome Prediction in Colombian University Students. Nutrients. 2017 Sep;9(9):1009.

15. Druet C, Ong KK. Early childhood predictors of adult body composition. Best Pract Res Clin Endocrinol Metab. 2008 Jun;22(3):489–502.

16. Labayen I, Moreno LA, Blay MG, Blay VA, Mesana MI, González-Gross M, et al. Early programming of body composition and fat distribution in adolescents. J Nutr. 2006;136(1):147–152.

17. Anderson AK. Association between Infant Feeding and Early Postpartum Infant Body Composition: A Pilot Prospective Study. Int J Pediatr. 2009;2009:1–7.

18. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. Being big or growing fast: systematic review of size and growth in infancy and later obesity. Bmj. 2005;331(7522):929.

19. Singhal A, Wells J, Cole TJ, Fewtrell M, Lucas A. Programming of lean body mass: a link between birth weight, obesity, and cardiovascular disease? Am J Clin Nutr. 2003;77(3):726–730.

20. Bakker I, Twisk JWR, Van Mechelen W, Kemper HCG. Fat-free body mass is the most important body composition determinant of 10-yr longitudinal development of lumbar bone in adult men and women. J Clin Endocrinol Metab. 2003 Jun;88(6):2607–13.

21. Prioreschi A, Munthali RJ, Kagura J, Said-Mohamed R, De Lucia Rolfe E, Micklesfield LK, et al. The associations between adult body composition and abdominal adiposity outcomes, and relative weight gain and linear growth from birth to age 22 in the Birth to Twenty Plus cohort, South Africa. PloS One. 2018;13(1):e0190483.

22. Navti LK, Ferrari U, Tange E, Bechtold-Dalla Pozza S, Parhofer KG. Contribution of socioeconomic status, stature and birth weight to obesity in Sub-Saharan

Africa: cross-sectional data from primary school-age children in Cameroon. BMC Public Health. 2014 Apr 7;14:320.

23. Elliott AM, Kizza M, Quigley MA, Ndibazza J, Nampijja M, Muhangi L, et al. The impact of helminths on the response to immunization and on the incidence of infection and disease in childhood in Uganda: design of a randomized, double-blind, placebo-controlled, factorial trial of deworming interventions delivered in pregnancy and early childhood [ISRCTN32849447]. Clin Trials Lond Engl. 2007;4(1):42–57.

24. Lule SA, Webb EL, Ndibazza J, Nampijja M, Muhangi L, Akello F, et al. Maternal recall of birthweight and birth size in Entebbe, Uganda. Trop Med Int Health. 2012 Dec;17(12):1465–9.

25. Lule A S, Namara B, Akurut H, Lubyayi L, Nampijja M, Akello F, et al. Are birth weight and postnatal weight gain in childhood associated with blood pressure in early adolescence? Results from a Ugandan birth cohort. Int J Epidemiol. 2018;

26. VanItallie TB, Yang MU, Heymsfield SB, Funk RC, Boileau RA. Height-normalized indices of the body's fat-free mass and fat mass: potentially useful indicators of nutritional status. Am J Clin Nutr. 1990 Dec;52(6):953–9.

27. Mattar L, Pichard C, Godart N, Melchior JC. Can birth weight predict later body composition in anorexia nervosa? Eur J Clin Nutr. 2012;66(8):964–967.

28. Leroy J. ZSCORE06: Stata module to calculate anthropometric z-scores using the 2006 WHO child growth standards [Internet]. 2011 [cited 2018 Dec 5]. (Statistical Software Components). Available from: https://econpapers.repec.org/software/bocbocode/s457279.htm

29. Ong KK, Loos RJF. Rapid infancy weight gain and subsequent obesity: systematic reviews and hopeful suggestions. Acta Paediatr Oslo Nor 1992. 2006 Aug;95(8):904–8.

30. Kagura J, Feeley ABB, Micklesfield LK, Pettifor JM, Norris SA. Association between infant nutrition and anthropometry, and pre-pubertal body composition in urban South African children. J Dev Orig Health Dis. 2012 Dec;3(6):415–23.

31. Harder T, Bergmann R, Kallischnigg G, Plagemann A. Duration of breastfeeding and risk of overweight: a meta-analysis. Am J Epidemiol. 2005 Sep 1;162(5):397–403.

32. Victora CG, Barros F, Lima RC, Horta BL, Wells J. Anthropometry and body composition of 18 year old men according to duration of breast feeding: birth cohort study from Brazil. Bmj. 2003;327(7420):901.

33. Holzhauer S, Hokken Koelega ACS, Ridder M de, Hofman A, Moll HA, Steegers EAP, et al. Effect of birth weight and postnatal weight gain on body composition in early infancy. Early Hum Dev. 2009 May;85(5):285–90.

34. Leary SD, Lawlor DA, Davey Smith G, Brion MJ, Ness AR. Behavioural early-life exposures and body composition at age 15 years. Nutr Diabetes. 2015 Feb 9;5(2):e150.

35. Beeson WL, Batech M, Schultz E, Salto L, Firek A, Deleon M, et al. Comparison of body composition by bioelectrical impedance analysis and dual-energy X-ray absorptiometry in Hispanic diabetics. Int J Body Compos Res. 2010;8(2):45–50.

Abstract

Background: The relationship between blood pressure (BP) and early-life factors such as birth weight, life-course factors such as obesity, and genetic variants among Africans are unknown. This PhD aimed to systematically review literature on the relationship between birth weight and BP among young Africans and to use data from the Entebbe Mother and Baby Study (EMaBS), a Ugandan birth cohort, to investigate the impact of early-life, life-course and genetic factors on adolescents' BP.

Methods: Four databases were systematically searched for relevant publications. In the EMaBS, data were collected prenatally from pregnant women and prospectively from the resulting offspring, with BP measured in adolescence. Linear regression was used to relate birth weight, early-growth and other characteristics to BP. Genetic analyses were conducted to investigate genetic variants associated with adolescents' BP.

Results: The systematic review showed that among the few published studies from Africa, the relationship between birth weight and BP varied by participants' age. Findings from the EMaBS indicated strong evidence of association between postnatal weight gain and later BP, with fast-growing low birth weight individuals particularly affected. Maternal factors positively associated with higher adolescent BP were gestational body mass index (BMI) and higher education status. Adolescent factors positively associated with higher BP were age, waist circumference, BMI, family history of hypertension and current *Trichuris* infection. Previous malaria infection in childhood and higher vegetable or fruit consumption were associated with lower BP in adolescence. No genetic variant reached genome-wide significance for association with BP. Thirty-three of 330 variants previously identified as associated with BP were replicated in this study, but none were significant after accounting for multiple testing.

Conclusions: Postnatal weight gain rather than birth weight is associated with later BP, with fast-growing low birth weight individuals at particular risk. Larger studies are required to characterise BP genetics in African adolescents.