Appendix 16

Entebbe Mother and Baby Study

PROTOCOL AMENDMENT

Amendment title: Blood pressure and cardiovascular disease risk factors among 10 and 11 year old children in the Entebbe Mother and Baby Study

4th September 2013

Investigators and collaborating institutions		
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Funding Organisations: Medical Research Council, Wellcome Trust

Contents

Table 1. Summary of new activities to be conducted as part of the new work, at age 10 to 11 years	
1.	Consent and assent for blood pressure study procedures
2.	Anthropometry (waist and upper arm circumference; weight and height are already measured routinely at annual visits)
3.	Detailed questionnaire and examination related to blood pressure and metabolic outcomes
4.	Blood pressure measurement
5.	Blood sample at age 10 or 11 years: 14 mls for lipid profiles (Cholesterol, HDL, LDL, Triglycerides), glucose, HbA1c, CRP
6.	Blood sample storage
6.	Urine sample collection and storage

1. Summary

Hypertension is a rapidly increasing health concern in Africa. Hypertension begins in childhood and contributes to early development of cardiovascular disease (CVD). High blood pressure in childhood is most commonly "essential hypertension" (has no specific identifiable cause) and in most cases in children it remains undiagnosed. Identifying and successfully managing children with high blood pressure may have important benefits for long term CVD outcomes.

The association between birth weight and blood pressure has been intensively studied in affluent countries but not in low-income settings. Our key goal is to investigate whether birth weight and pre- and peri-natal exposures are important in programming blood pressure in children in Uganda. In addition, we will assess the relationship between such exposures and other biomarkers of metabolic processes and CVD risk. Also, DNA archive and genetic data are already available for the cohort and this will allow us to analyse associations between genetic polymorphisms and blood pressure, and between genetic polymorphisms and biomarkers of CVD risk, in this population.

The existence of the Entebbe Mother and Baby Study (EMaBS) birth cohort offers a unique opportunity to investigate these associations, providing information on the likely aetiology of high blood pressure and of metabolic disorders, and enabling the generation of strategies for prevention and management

of cardiovascular disease for this population, and for similar settings. This study will be conducted among 10 and 11 year old children under follow up in the EMaBS.

2. Background

Hypertension is a major public health burden globally, and a rapidly growing one in Sub-Saharan Africa where at least 79 million people are believed to be affected (1). Overall, the prevalence of hypertension in Africa is estimated to range from 6 to 48% (2) but there is little or no reliable data on the prevalence of Non Communicable Diseases (NCD) and their associated risk factors in Africa (2-4). There is little attention to the prevention and management of hypertension in Uganda and similar countries in SSA, and, until recently, this has not been considered a public health priority.

The majority of the people affected by hypertension are unaware of it, and of those that are aware, many are not on treatment (4-6). Poor healthcare provision and late detection remain major obstacles to preventing and controlling hypertension in Sub Saharan Africa SSA (6). Studies in Uganda have shown a prevalence of hypertension of 22.5% in adults in rural Uganda (7) and of 28% among HIV-positive individuals on antiretroviral therapy(8), comparable to the prevalence of hypertension in developed countries (9, 10). Based on a United States normogram (11) (since local standards are not available) our pilot study in Wakiso District found blood pressure above the 95th centile in 17% of school children on the first reading. Despite the evidence of a growing burden of hypertension in SSA, much remains to be done to determine the extent of the problem, especially in children.

High blood pressure in childhood is a risk factor for adult hypertension

Hypertension, which contributes to cardiovascular disease, mortality and morbidity in adulthood (12-16), has its origins in childhood (17-19). Children with high blood pressure tend to be hypertensive adults (20). Despite this knowledge, very little attention has been paid to the problem of high blood pressure in children and adolescents, especially in developing countries.

Risk factors for hypertension and cardiovascular disease in developed countries

In developed countries, high blood pressure is linked to low birth weight (21-25), prematurity (26), body mass index (BMI) (16, 27-29) gender (29, 30), ethnicity (30) and waist circumference (WC) (28). Also, among low birth weight infants, accelerated weight gain is associated with elevated blood pressure (21-23, 31). Other important biomarkers for cardiovascular disease risk – for example elevated blood

glucose, elevated triglycerides, reduced high density Lipids (HDL) cholesterol – are often associated with high blood pressure in the metabolic syndrome (24, 32). Also, there is increasing evidence of a link between the metabolic syndrome and chronic, low-grade inflammation, assessed (for example) by circulating C-reactive protein (CRP) levels. C-reactive protein levels show additive effects for cardiovascular disease over and above the metabolic risk factors (32).

Risk factors for high blood pressure and cardiovascular disease risk factors in childhood in Africa

Data on risk factors for high blood pressure in childhood in Africa are very limited (33-35). In Ghana, increased body mass index (BMI) and urbanisation were risk factors for high blood pressure among school children (35). Low socio-economic status was linked to elevated blood pressure in Kinshasa (34). However, these studies were based on a single blood pressure reading, giving limited accuracy.

Other risk factors for cardiovascular disease may be strongly influenced by environmental factors in our setting. For example, CRP levels in adults – noted above as a marker of low grade inflammation and CVD risk - have been shown to be markedly lower among adults in rural Ecuador, or in the Philippines, when compared with adults in the United States, and there is evidence that this may be related to immunomodulation resulting from early-life infectious exposures (36). Similarly, there is recent evidence from animal models that helminth infections may influence glucose metabolism through their impact on the immune system: eosinophils, which are strongly induced by helminth infections, seem to influence macrophages in adipose tissue to adopt an anti-inflammatory profile, resulting in reduced insulin resistance and improved glucose tolerance (37). The Entebbe Mother and Baby Study, with its detailed data on helminth infections and their treatment, offers a unique opportunity to investigate whether helminth exposure in early life influences metabolic parameters, including CVD risk factors, in humans.

Relationship between birth weight and blood pressure in Africa

Among populations of African descent, the association between birth weight and blood pressure is not certain (38). In Soweto, South Africa (39), and Harare, Zimbabwe (40), birth weight was inversely associated with systolic blood pressure among school children. But in rural Gambia, birth weight was not associated with blood pressure (41). This could be because the causes of low birth weight differ between rural, tropical Africa and developed or sub-tropical settings: for example, malaria is an important cause of low birth weight which is restricted to the tropics (42). Alternatively, effects of the prenatal exposures associated with low birth weight may be modified by environmental factors in

infancy. In order to understand the conflicting results from studies described, there is a need to investigate effects of birth weight on blood pressure in an African setting, with heavy exposure to parasitic and other infectious diseases. The epidemiology of hypertension in tropical Africa may be different to that in the industrialised countries.

Genetics of hypertension and cardiovascular disease risk

Besides environmental influences, there is strong evidence that genetic traits contribute to hypertension (43). Meta-analyses of studies in Europeans and East Asians have identified single nucleotide polymorphisms related to the serine/threonine kinase 39 (STK39) gene and hypertension. However, these findings have not been replicated among people of African descent (44).

Genetic studies of complex traits in African subjects are important. Because African populations are older, genetic diversity is higher, and linkage disequilibrium (the tendency for groups of genes to be inherited together) is lower, among Africans than among Caucasians. Also, selection pressure driven by exposure to infections differs between these populations, exemplified by the striking observation that mutations in the apolipoprotein L1 gene in African Americans both protect against infection with Trypanosoma brucei rhodesiense and associate with an increased risk of developing renal glomerulosclerosis and hypertension (45).

Studies among African populations therefore have the potential to provide unique insights into genetic mechanisms behind human health and disease. Currently, an on-going study in Kyamulibwa, South West Uganda, a collaboration between the MRC/UVRI Unit and the Wellcome Trust Sanger Institute, is investigating genetic factors associated with hypertension and metabolic markers of cardiovascular disease risk among more than 5000 participants from a rural Ugandan population. At the same time, under the already-approved EMaBS amendment, Appendix 13 (“The genetics of response to vaccination, infectious and inflammatory disease in the children from the Entebbe Mother and Baby Study”) a genome-wide genetic analysis is being conducted at the Sanger Institute for samples from EMaBS, as part of a collaboration with the University of Oxford. This provides the opportunity for us now to examine genetic associations with blood pressure and biomarkers of CVD risk in EMaBS, and also to relate our findings to the results from the Kyamulibwa study.

Blood pressure and biomarkers of cardiovascular disease risk in the EMaBS

Within EMaBS, we now, therefore plan to investigate the influence of birth weight, of pre- and peri-natal exposures and of genetic polymorphisms, on blood pressure and on metabolic parameters, particularly those associated with CVD risk, at school age in a birth cohort in the tropics. The EMaBS birth cohort represents a unique opportunity to investigate these associations among Ugandan children aged 10 and 11 years. Data on birth weight and on infectious exposures has been collected since pregnancy, through labour, delivery, infancy to childhood. This will help us to understand whether birth weight and pre- and peri-natal exposures are important in programming blood pressure and CVD risk factors among African children.

3. Rationale for the study

Little or no research has been conducted on childhood blood pressure in Uganda and it is likely that hypertension will be an increasing health problem as a result of the societal transition. This work is one of a number of studies on blood pressure and on the metabolic syndrome that the MRC/UVRI hopes to conduct over the forthcoming years in Uganda.

EMaBS is one of the few birth cohorts that have been established in tropical Africa. It offers a unique opportunity to study the influence on blood pressure at school age of birth weight, and of pre- and peri-natal exposures, in the tropics. In addition, genetic data are being generated for cohort members under an approved, earlier, protocol amendment and will be available to allow analysis of genetic markers associated with blood pressure in this population.

The existence of data from this large birth cohort of mothers and babies is a rare opportunity to investigate the influence of birth weight, and of pre- and peri-natal exposures, on blood pressure at school age in the tropics. Identifying and successfully treating children with high blood pressure may have an important impact on long term CVD outcomes. This is a society in transition and this work will allow us to inform the Ministry of Health on the burden of high blood pressure at an early age. Understanding the risk factors for hypertension will be essential in mitigating the effects of this burgeoning epidemic, and will provide data for formulating evidence-based health policy and interventions. This may have global implications in terms of the design of interventions again hypertension and cardiovascular disease.

4. Hypothesis

The fetal origin hypothesis proposes that intra uterine nutrition and growth, of which birth weight is a key marker, are vital in programming blood pressure in later life. The rapid growth hypothesis suggests that the adverse effects of low birth weight are further strengthened by a rapid increase in weight after birth, and that this, too, influences blood pressures in later life. These risk factors have been intensively studied in affluent countries but not in low-income settings, where infectious diseases are the leading cause of morbidity and mortality, including low birth weight. However the burden of non-communicable diseases and of hypertension in particular, is rapidly growing in Africa, while the continent is ill-equipped to address this burden. We will address the hypothesis that birth weight and pre- and peri-natal infectious exposures are important in programming blood pressure and metabolic factors associated with cardiovascular disease risk among children in the tropics.

5. Objectives

The key goal is to investigate whether birth weight and other pre- and peri-natal exposures are important in programming blood pressure in children

5.1. Specific objectives

5.1.1. To determine whether there is an association between birth weight and blood pressure among 10 and 11 year old children in EMaBS.

5.1.2. To identify other early life exposures associated with blood pressure among 10 and 11 year old children in EMaBS.

5.1.3. To investigate whether the same exposures are associated with metabolic parameters linked to CVD risk among 10 and 11 year old children in EMaBS.

5.1.4. To investigate whether genetic polymorphisms are associated with blood pressure and with metabolic parameters linked to CVD risk among 10 and 11 year old children in EMaBS.

6. Methods

6.1. Study design

This is a longitudinal study that will take advantage of the already existing EMaBS birth cohort under follow up in Entebbe Uganda. The study will be conducted in Entebbe at the EMaBS study clinic and in the MRC/UVRI Laboratories. The participant data collected at the 10 or 11 year old study visit will be linked to earlier individual data that has been collected from pregnancy through delivery to date, and to genetic data that has been obtained through a previously-approved EMaBS protocol amendment.

6.2. Study area and participants

This study will be conducted among the current EMaBS participants. Briefly, the EMABS is located on the Entebbe peninsula in Uganda, an area occupied by peri-urban, rural, and fishing communities. In total, 2507 pregnant women were enrolled and 2345 live births were recorded (46, 47). There are 1964 (82 %) children for whom birth weight is available (46) and 1622 (69%) children were seen at age 5 years (48). We continue to see over 1000 children for routine follow up visits per year.

Children aged 10 or 11 years of age who are currently under follow up in the EMaBS birth cohort will be invited to enrol into this study. They will be included in the study at routine annual visits if they are well, in the opinion of the study physician and if written, informed consent is provided by the parent or guardian, and written, informed assent is provided by the child. Children who are unwell will be treated for current illness and invited to return for the blood pressure assessment when they have recovered. Children will be excluded from this assessment if consent and assent for it are not given.

6.3. Outcome measures

6.3.1. **The primary outcomes will be systolic and diastolic blood pressure at age 10-11 years.** This age range has been chosen to allow all active cohort participants to be seen within a two-year period between early 2014 and early 2016. This is the period for which funding for this study is available.

6.3.2. **Secondary outcomes will be lipid profiles, glycosylated haemoglobin A1c (HbA1c) and CRP levels measured in a random (i.e. non-fasting) blood sample at age 10-11 years.** Lipid profiles will include total cholesterol and high density lipoprotein (HDL)

levels. HbA1c measurement will provide an estimate of integrated plasma glycaemia for the previous 2-3 month (49, 50). CRP will be measured to assess chronic inflammation. If funds permit, CRP will be assessed not only at 10/11 years, but also in plasma or serum samples stored at annual visits from age one to nine years, for exploratory analyses investigating the hypothesis that patterns of chronic, low-grade inflammation are established early in life. In addition, a sample of urine and a sample of plasma or serum will be stored for investigation using "metabolomics"(see below) if additional funding for this can be secured.

6.4. Exposures

6.4.1. Birth weight (grams) will be the main exposure

Other exposures considered will include the following:

6.4.2. Child's gender, and age at the time of blood pressure assessment

6.4.3. Household and family risk factors (socio-economic status, environmental factors in the home, location of residence, parental education, tribe, sleep, family history of high blood pressure, heart disease and stroke.)

6.4.4. Life-course exposures (prenatal exposure to maternal helminth infections and their treatment, and other infections during pregnancy; helminth infections and treatment, and other infectious disease episodes during infancy; childhood, length and head circumference at birth, weight and height trajectory during infancy and childhood)

6.4.5. Current anthropometric parameters: height, weight and waist circumference

6.4.6. Genetic markers

6.5. Study procedures

Provisions of the existing EMaBS protocol include routine annual visits at which a questionnaire is completed, weight and height are measured, a stool sample is obtained and albendazole treatment is provided as a routine to all participants. The additional procedures required for this blood pressure study will be conducted alongside the already-approved procedures. Parents or guardians are often contacted before the visit, to remind them to attend, and for this visit they will be asked to bring the child in the morning, fasting, if possible.

Appendix 16

After explaining the procedures for the blood pressure study (BPS) and obtaining consent and assent for the additional BPS procedures, anthropometric measurements for height, weight and the waist circumference (WC) will be taken when the child is wearing light clothing. The WC will be measured at the mid-way position between the lowest rib and the iliac crest and recorded to the nearest 0.1 cm. The weight will be measured to the nearest 1 kg and height at the nearest 0.1 cm. A study questionnaire will be completed by the study investigator with the help of the parent or guardian. The time since food or drink was last taken will be recorded.

The child will be asked to sit and rest for at least five minutes. Then systolic and diastolic blood pressure will be measured using the appropriate cuff size for the arm circumference (51).Three readings will be taken five minutes apart. The average blood pressure reading for that visit will be compared with international normograms (52), for gender by age and height percentile (Appendix 1). A child with an average blood pressure $\geq 95^{th}$ centile based on their gender, age and height percentile will be invited for two more visits on different days for additional blood pressure measurement. Hypertension is defined as an average systolic and or/ diastolic blood pressure that is $\geq 95^{th}$percentile for gender, age and height on three separate occasions (51, 52). The height percentile will be determined using the standard growth chart (51).

Blood tubes will be labelled with a unique identification number and date of collection, linking the sample to the individual child data already collected. The total volume of blood collected will be 14 mls: 6mls of blood will be collected in an EDTA tube, 4mls in the fluoride oxalate tube and 4 mls in a serum tube, from each child on the first visit. From the EDTA tube, a blood smear for malaria parasites will be made and read and 2mls will be removed for measurement of Full Blood Count (FBC) and for investigation for *M. perstans* infection. Blood will be aliquoted into labelled microtubes and stored at –80 ^{0}C for later measurement for glycosylated haemoglobin (HbA1c). Serum samples will be stored for measurement of urea and electrolytes among children found to have high blood pressure, and for lipid profile and CRP. The 4mls in the fluoride oxalate tube will be used to measures fasting/random blood sugars. After the blood sample has been taken the children will be provided with refreshments such as juice, biscuits, fruit.

High blood pressure will be categorised according to the extent to which a child's blood pressure exceeds the 95th centile (51). Children with hypertension will be investigated for serum urea and electrolytes and glomerular filtration rate, urine dipstick for proteinuria, renal ultrasound, chest x-ray,

electrocardiogram and echocardiogram. They will be managed for hypertension in consultation with a paediatric blood pressure specialist, Dr Amos Odiit (collaborator) at Mulago Hospital.

Measurement of blood pressure will be introduced as part of the annual visit routine for all children aged above 10/11 years. This will allow monitoring of those with pressures that are elevated but to a lesser degree.

6.6. Laboratory investigations

A portion of the blood sample will be used for a full blood count, and will be examined for malaria by thick and thin film, and for *Mansonella perstans* infection using a modified Knott's method. Individuals found to have positive results for malaria will be treated according to government guidelines (currently with artemether combination therapy as first line), whether or not they are symptomatic. Lipid profiles (Cholesterol, High Density Lipids (HLD), Low Density Lipids (LDL) and Triglycerides) will be measured using the homogeneous enzymatic colorimetric assay.

The remaining blood will be aliquoted into labelled microtubes and stored at – 80 ^{0}C for later measurement for HbA1c using Turbidimetric inhibition immunoassay. Plasma/serum will be stored for later measurement of CRP.

6.7. Plasma/serum and urine will also be stored for metabolomics. Metabolomics involves the measurement and profiling of small molecules that can reflect the metabolic response at the cellular, tissue and organ level and can provide us with further insight into the functioning and induction of the immune response. Metabolomic studies in infection models have revealed novel insight into how lipid related metabolites can play an important role in viral (53) and bacterial (54) infections. Importantly, exciting work has shown that the metabolic state of immune cells can have far reaching consequences for the development of long term memory response (55). Of particular relevance to this project, there is also evidence that helminth infections can influence immune responses and inflammation through production of small molecules such as short chain fatty acids (acetate, proprionate and butyrate), which have immunomodulating properties (56, 57). Therefore, whole metabolome measurements will be made in urine and serum and both untargeted and pathway specific analysis will be applied to identify metabolic profiles related to infectious exposure and CVD risk factors. This work will be conducted in collaboration with colleagues at the Leiden University Medical Centre, the Netherlands, where suitable equipment and expertise is available. A further protocol amendment with details of the methods to be used and analyses to be conducted, and a material transfer agreement, will be submitted when funding for this work has been secured.Sample size determination

The sample size is determined by the number of children still under follow-up within the cohort. We are aiming to see at least 1000 children. This will give considerable power to detect effects even of quite

rare exposures on the primary outcome, blood pressure. For example, assuming 8% were low birth weight (47) and that the standard deviation among children aged 10 years is 12 mmHg for systolic blood pressure and 8 mmHg for diastolic blood pressure (as in our recent pilot study in schools – unpublished data) this study will have over 80% power, with p=0.05, to detect an elevation of systolic blood pressure of 4 mm Hg and nearly 90% power to detect and elevation of diastolic blood pressure among low birth weight children of 3 mmHg. However using birth weight as a continuous explanatory variable will give greater power to detect effect on blood pressure than dichotomising birth weight (58).

A cohort of 1000 infants with the same measured variability in blood pressure would have 80 % power to detect an associated genetic variant with a mean allele frequency of 10 % producing a change in blood pressure of 4 mmHg to a GWAS level of significance (5×10^{-8}) using an additive mode of inheritance (calculated using the QUANTO software**: http://hydra.usc.edu/gxe).** Even if GWAS significant associations are not found within this cohort, it would be possible to use findings both from previously published work (59) and unpublished work from the Kyamulibwa analyses to model contributions of genetic variants and other measured variables on the blood pressure outcome (60).

6.8. Ethical considerations

The influence of birth weight on blood pressure among African children is still unknown and this is what this study will investigate.

Blood pressure assessment carries no risk to the child and causes only very minor discomfort. Obtaining a blood sample causes minor discomfort. The participants will be informed of the results of their blood pressure assessment, and of results from the blood tests as they become available. This is likely to have health benefits, including education about high blood pressure for those with normal findings, and education, provision of investigation, and advice and assistance with management for those found to be affected. Treatment will be offered, if required, up to the end of our current funding period (expected to be August 2016). Affected children and their parents or guardians will be referred for further management at Entebbe Hospital if follow up at the EMaBS clinic is not possible thereafter. Entebbe Hospital Staff will be offered continuing medical education on the management of high blood pressure in children to enable them to take this on.

Genetic data are already available for children in EMaBS, based on approval previously given for protocol Appendix 14. The informed consent process for this study will include an explanation that we now propose to use the genetic data and available DNA to examine associations with blood pressure.

Collected data will be securely and confidentially kept by the statistician/ data manager.

EMaBS children each have a unique identifier which is used at all study visits and on all data collection forms. The unique identifier, unavoidably, has the potential to allow data to be linked to an individual child. The unique identifier will be used to create the required, merged data sets for this analysis. These will include socio-demographic and clinical data from the current, and previous, study visits, laboratory data on infections, laboratory data on CVD biomarkers and genetic data. Data shared between collaborators in the study will be anonymised. Genetic data made available in open access, on-line databases will have all identifiers removed.

Both written informed consent and assent will be obtained for each child's participation in the additional blood pressure study procedures, and for the additional genetic analyses. Ethical approval will be sought from the ethics committees the Uganda Virus research Institute Ethics committee and the Uganda National Council for Science and Technology.

6.9. Data management

6.9.1. Data entry and quality assurance

Data will be collected on standardised clinical record forms (CRFs) by trained nurses, clinician and laboratory personnel. Sample identification data will be recorded on these forms at the time of sample collection, and results will be added at the laboratory. Completed CRFs will be collated and batched by the research teams or the Study Clerk, and then sent to the MRC/UVRI statistics section for double data entry and verification by data entrants.

The statistician / data manager will review entered data and generate queries at regular intervals which will be addressed by field, clinic or laboratory staff. Necessary corrections will be made. Data entry and data management will be overseen by a statistician/data manager at the MRC Unit. Data will be maintained on the MRC Unit server and backed up using standard Unit procedures.

Genetic data will be generated and analysed predominantly in the laboratories of the collaborating institutions: Oxford and the Sanger Institute. Plans are in place to develop sufficient infrastructure to

perform complex genetic analyses, such as those proposed herein, within Entebbe, Uganda. Blanket consent for genetic analyses related to infectious and inflammatory disease has already been approved and acquired using the EMaBS amendment 13. The re-analysis of the genetic data with respect to hypertension and other related cardiovascular disease will be stressed to the participant during the consenting process for this new study. Again, in line with the EMaBS amendment 13 and recent guidelines released by the European Society of Human Genetics (61). If any deleterious variants are identified and validated in any participant, every effort will be made to re-contact the individual concerned and provide appropriate ethical counselling. Such steps would only be taken after close consultation with experts in clinical genetics at the collaborating genetic institutions and following any additional guidelines that may be in place at the time. Such re-contacting would be facilitated by the excellent network in place as part of the on-going EMaBS. All resultant data will also be deposited in international repositories such as the European Bioinformatics Institute in Hinxton, UK in keeping with routine procedures and the previous ethical approval of genetic analyses undertaken for EMaBS. Any such storage will be anonymised using different identifying codes to those used within EMaBS.

6.9.2. Analysis plan

For each study objective, the analysis plan has been set out. Prior to each analysis, a list of all variables to be considered will be prepared.

6.9.2.1. To determine whether there is an association between birth weight and blood pressure among 10 and 11 year old children in EMaBS.

Blood pressure will be treated as a continuous variable. We will conduct univariable analyses to assess the relationship between birth weight and blood pressure. We will fit multiple regression models of blood pressure separately for systolic and diastolic, on birth weight and appropriate potential confounders.

6.9.2.2. To identify other early life exposures associated with blood pressure among 10 and 11 year old children in EMaBS.

Univariable analyses for early life exposures in utero and early life will be conducted. We will fit multiple regression models of blood pressure separately for systolic and diastolic pressure on early life exposures and appropriate potential confounders.

We will develop a conceptual framework describing anticipated relationships between the exposures that we have assessed. Based on these, univariable and multivariable analyses models will be fitted to assess the relationships between the main exposures, the potential confounders, and the primary and secondary outcomes.

6.9.2.3. To investigate whether the same exposures are associated with metabolic parameters among 10 and 11 year old children in EMaBS.

Univariate and multiple regression models will be fitted for each metabolic parameter (HbA1c, HDL and total cholesterol) on potential risk factors and confounders.

6.9.2.4. To investigate whether genetic polymorphisms are associated with blood pressure and biomarkers of CVD risk among 10 and 11 year old children in EMaBS.

Genome-wide genotype data at 2.5 million variant sites will be available for a large proportion of the EMaBS children through the prior-approved GWAS study. As part of the original analysis the data will have already undergone significant quality control procedures including exclusion of individuals based on low call rates and high rates of heterozygosity, and genotyped SNPs will have been excluded based on high rates of missingness and heterozygosity and deviation from Hardy-Weinburg equilibrium. Each genetic variant will be tested for association with blood pressure both as a quantitative and binary variable using linear or logistic regression accounting for the measured covariates discussed above. Ethnicity will be controlled for using a mixed model (GEMMA) analysis or similar. If no significant associations are identified, then established methods will be used to test for contribution of recognised associated variants in the development of pathological hypertension using established methodology (60).

Furthermore, a range of other analyses will be possible using the existing data and material. For example, it will be possible to test for association of variants within genes such as ApoL1 using a gene association method of analysis or it may be possible to identify variants associated with the development of extreme phenotypes of hypertension using either the existing genotype data or next-generation sequencing techniques such as exome or whole-genome sequencing which are planned for the existing EMaBS cohort genetic studies.

7. Quality assurance and audit

To ensure quality and reliability of the data of this study SOPs will be developed for clinic and laboratory procedures. The study will be regular monitored and conducted in accordance to the principles of GCP/GCLP.

8. Potential limitations; anticipated problems

Some data of interest have not yet been collected, such as paternal details in relation to hypertension, such as family history, which may be important. It will only be possible to collect this information from children who are still under follow up (and even for some of those under follow up, information about their parents may be lacking). For mothers, blood pressure reading at enrolment during pregnancy is available, as well as history of hypertension in the mother's family.

9. Significance of the proposed work

This will be the first study in Uganda, and one of the first in the region, to investigate whether birth weight and pre- and peri-natal exposures are important in programming blood pressure among African children. The existence of this long standing birth cohort in Entebbe is a unique opportunity to investigate factors associated with blood pressure programming in an African setting. The study will provide data on important exposures associated with high blood pressure in children. This will be important in developing strategies for prevention of hypertension and cardiovascular disease in this setting.

10. Distribution of responsibilities

The Principal Investigator will be responsible for overseeing all aspects of the study. The Study team will consist of the Principal Investigator, other co-investigators, the study physicians, clinical and laboratory staff, as well as field workers/ interviewer who will regularly meet to discuss any study related issues, including medical and administrative issues. The Principal Investigator and project leader will prepare and submit annual progress reports to ethics committees.

11. Plan for dissemination

The MRC Unit has strong links the Ministry of Health, and has been requested to assist with research on non-communicable diseases in Uganda. This close contact will enable us to disseminate our results to

policy makers in Uganda. Also, we have a long-standing tradition within the Entebbe community and through participant meetings we will continue to inform the community of our findings. The academic community will be informed through local and international scientific meetings and the publication of papers in relevant peer reviewed journals.

12. Budget

12.1. Logistics and estimated cost

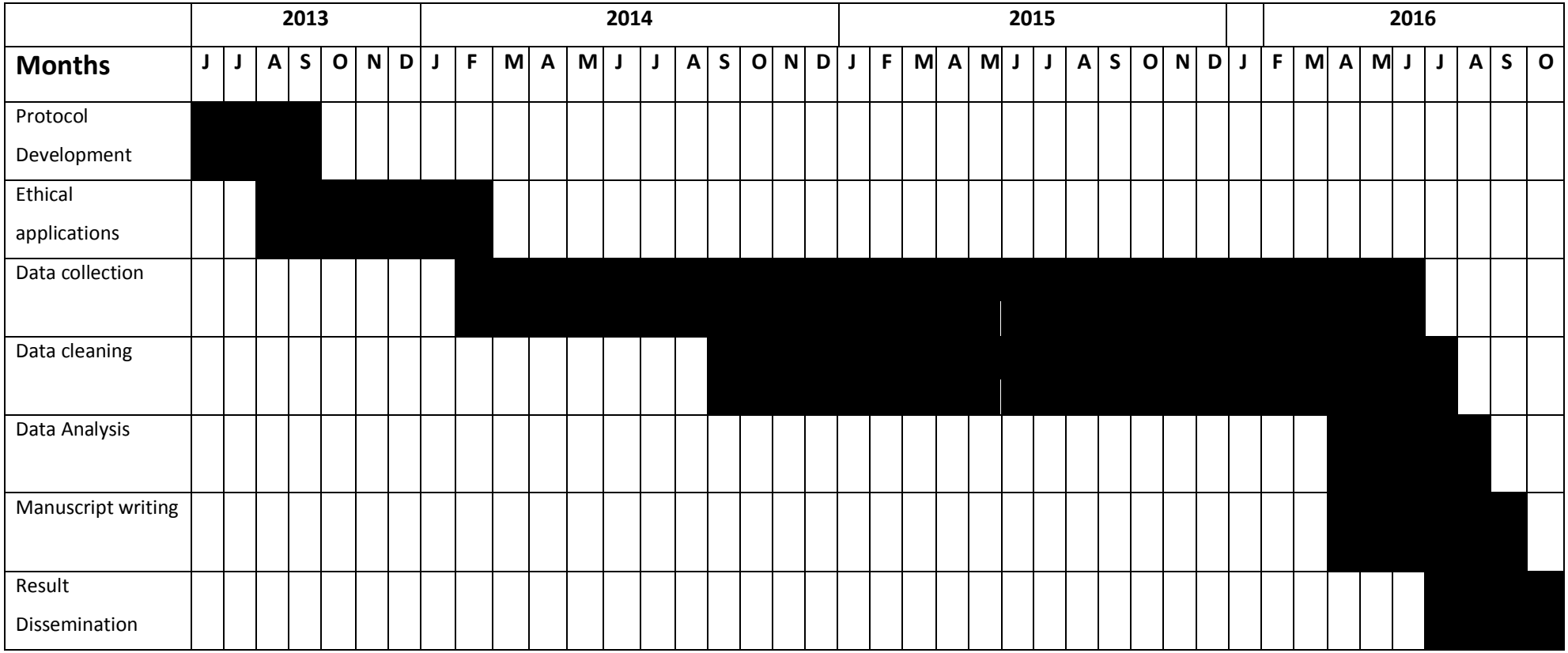
The Entebbe Mother and Baby Study is supported by an on-going Wellcome Trust fellowship held by Professor Elliott. The supplementary funding required for this blood pressure study will be provided from MRC Unit core funds. The budget is as follows:

Item	**Costs**
Salaries	£37,158
Statistical support	£600
Transport costs	£3,980
Laboratory tests	£15,180
Other costs	£11,289
Total Cost	£68,207

Appendix 16

12.2. Timeframe

This is the proposed time frame for the blood pressure study



13. References

1. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet. 2005 Jan 15-21;365(9455):217-23. PubMed PMID: 15652604. Epub 2005/01/18. eng.
2. 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. International journal of epidemiology. 2011 Aug;40(4):885-901. PubMed PMID: 21527446. Epub 2011/04/30. eng.
3. Namusisi O, Sekandi JN, Kasasa S, Wasswa P, Kamara NT, Bitekyerezo M, et al. Risk factors for non-communicable diseases in rural Uganda: a pilot surveillance project among diabetes patients at a referral hospital clinic. The Pan African medical journal. 2011;10:47. PubMed PMID: 22384293. Pubmed Central PMCID: 3290877. Epub 2011/01/01. eng.
4. Mufunda J, Chatora R, Ndambakuwa Y, Nyarango P, Kosia A, Chifamba J, et al. Emerging non-communicable disease epidemic in Africa: preventive measures from the WHO Regional Office for Africa. Ethnicity & disease. 2006 Spring;16(2):521-6. PubMed PMID: 17682258. Epub 2007/08/08. eng.
5. Ibrahim MM, Damasceno A. Hypertension in developing countries. Lancet. 2012 Aug 11;380(9841):611-9. PubMed PMID: 22883510. Epub 2012/08/14. eng.
6. Addo J, Smeeth L, Leon DA. Hypertension in sub-saharan Africa: a systematic review. Hypertension. 2007 Dec;50(6):1012-8. PubMed PMID: 17954720. Epub 2007/10/24. eng.
7. Maher D, Waswa L, Baisley K, Karabarinde A, Unwin N, Grosskurth H. Distribution of hyperglycaemia and related cardiovascular disease risk factors in low-income countries: a cross-sectional population-based survey in rural Uganda. Int J Epidemiol. 2010 Oct 5. PubMed PMID: 20926371. Epub 2010/10/12. Eng.
8. Mateen FJ, Kanters S, Kalyesubula R, Mukasa B, Kawuma E, Kengne AP, et al. Hypertension prevalence and Framingham risk score stratification in a large HIV-positive cohort in Uganda. Journal of hypertension. 2013 Apr 23. PubMed PMID: 23615323. Epub 2013/04/26. Eng.
9. Bertoia ML, Waring ME, Gupta PS, Roberts MB, Eaton CB. Implications of new hypertension guidelines in the United States. Hypertension. 2012 Sep;60(3):639-44. PubMed PMID: 22868391. Epub 2012/08/08. eng.
10. Ong KL, Cheung BM, Man YB, Lau CP, Lam KS. Prevalence, awareness, treatment, and control of hypertension among United States adults 1999-2004. Hypertension. 2007 Jan;49(1):69-75. PubMed PMID: 17159087. Epub 2006/12/13. eng.
11. National High Blood Pressure Education Program Working Group on High Blood Pressure in C, Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004 Aug;114(2 Suppl 4th Report):555-76. PubMed PMID: 15286277.
12. Pastor-Barriuso R, Banegas JR, Damian J, Appel LJ, Guallar E. Systolic blood pressure, diastolic blood pressure, and pulse pressure: an evaluation of their joint effect on mortality. Annals of internal medicine. 2003 Nov 4;139(9):731-9. PubMed PMID: 14597457. Epub 2003/11/05. eng.
13. Antikainen RL, Jousilahti P, Vanhanen H, Tuomilehto J. Excess mortality associated with increased pulse pressure among middle-aged men and women is explained by high systolic blood pressure. Journal of hypertension. 2000 Apr;18(4):417-23. PubMed PMID: 10779092. Epub 2000/04/25. eng.
14. Almgren T, Persson B, Wilhelmsen L, Rosengren A, Andersson OK. Stroke and coronary heart disease in treated hypertension -- a prospective cohort study over three decades. Journal of internal medicine. 2005 Jun;257(6):496-502. PubMed PMID: 15910553. Epub 2005/05/25. eng.

15. Antikainen R, Jousilahti P, Tuomilehto J. Systolic blood pressure, isolated systolic hypertension and risk of coronary heart disease, strokes, cardiovascular disease and all-cause mortality in the middle-aged population. Journal of hypertension. 1998 May;16(5):577-83. PubMed PMID: 9797168. Epub 1998/10/31. eng.
16. Falkner B. Hypertension in children and adolescents: epidemiology and natural history. Pediatr Nephrol. 2010 Jul;25(7):1219-24. PubMed PMID: 19421783. Pubmed Central PMCID: 2874036. Epub 2009/05/08. eng.
17. Berenson GS, Srinivasan SR, Bao W, Newman WP, Tracy RE, Wattigney WA, et al. Association between Multiple Cardiovascular Risk Factors and Atherosclerosis in Children and Young Adults. N Engl J Med. 1998 June 4, 1998;338(23):1650-6.
18. Lauer RM, Clarke WR. Childhood risk factors for high adult blood pressure: the Muscatine Study. Pediatrics. 1989 Oct;84(4):633-41. PubMed PMID: 2780125. Epub 1989/10/01. eng.
19. McNiece KL, Poffenbarger TS, Turner JL, Franco KD, Sorof JM, Portman RJ. Prevalence of hypertension and pre-hypertension among adolescents. The Journal of pediatrics. 2007 Jun;150(6):640-4, 4 e1. PubMed PMID: 17517252. Epub 2007/05/23. eng.
20. Flynn JT. Pediatric hypertension update. Current opinion in nephrology and hypertension. 2010 May;19(3):292-7. PubMed PMID: 20164767. Epub 2010/02/19. eng.
21. 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. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2002 Dec;26(12):1539-46. PubMed PMID: 12461670. Epub 2002/12/04. eng.
22. Adair LS, Martorell R, Stein AD, Hallal PC, Sachdev HS, Prabhakaran D, 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? The American journal of clinical nutrition. 2009 May;89(5):1383-92. PubMed PMID: 19297457. Pubmed Central PMCID: 2720838. Epub 2009/03/20. eng.
23. Law CM, de Swiet M, Osmond C, Fayers PM, Barker DJ, Cruddas AM, et al. Initiation of hypertension in utero and its amplification throughout life. BMJ. 1993 Jan 2;306(6869):24-7. PubMed PMID: 8435572. Pubmed Central PMCID: 1676382. Epub 1993/01/02. eng.
24. Blake KV, Gurrin LC, Evans SF, Beilin LJ, Stanley FJ, Landau LI, et al. Adjustment for current weight and the relationship between birth weight and blood pressure in childhood. Journal of hypertension. 2000 Aug;18(8):1007-12. PubMed PMID: 10953990. Epub 2000/08/23. eng.
25. Pharoah PO, Stevenson CJ, West CR. Association of blood pressure in adolescence with birthweight. Archives of disease in childhood Fetal and neonatal edition. 1998 Sep;79(2):F114-8. PubMed PMID: 9828737. Pubmed Central PMCID: 1720839. Epub 1998/11/26. eng.
26. Keijzer-Veen MG, Dulger A, Dekker FW, Nauta J, van der Heijden BJ. Very preterm birth is a risk factor for increased systolic blood pressure at a young adult age. Pediatr Nephrol. 2010 Mar;25(3):509-16. PubMed PMID: 20012998. Pubmed Central PMCID: 2810359. Epub 2009/12/17. eng.
27. Hansen ML, Gunn PW, Kaelber DC. Underdiagnosis of Hypertension in Children and Adolescents. JAMA. 2007 August 22, 2007;298(8):874-9.
28. Bekkers MB, Brunekreef B, Koppelman GH, Kerkhof M, de Jongste JC, Smit HA, et al. BMI and waist circumference; cross-sectional and prospective associations with blood pressure and cholesterol in 12-year-olds. PloS one. 2012;7(12):e51801. PubMed PMID: 23251628. Pubmed Central PMCID: 3522600. Epub 2012/12/20. eng.
29. Sorof JM, Lai D, Turner J, Poffenbarger T, Portman RJ. Overweight, ethnicity, and the prevalence of hypertension in school-aged children. Pediatrics. 2004 Mar;113(3 Pt 1):475-82. PubMed PMID: 14993537. Epub 2004/03/03. eng.

30. Urrutia-Rojas X, Egbuchunam CU, Bae S, Menchaca J, Bayona M, Rivers PA, et al. High blood pressure in school children: prevalence and risk factors. BMC pediatrics. 2006;6:32. PubMed PMID: 17109750. Pubmed Central PMCID: 1657006. Epub 2006/11/18. eng.
31. Walker SP, Gaskin P, Powell CA, Bennett FI, Forrester TE, Grantham-McGregor S. The effects of birth weight and postnatal linear growth retardation on blood pressure at age 11-12 years. Journal of epidemiology and community health. 2001 Jun;55(6):394-8. PubMed PMID: 11350995. Pubmed Central PMCID: 1731923. Epub 2001/05/15. eng.
32. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation. 2003 Jan 28;107(3):391-7. PubMed PMID: 12551861.
33. Monyeki KD, Kemper HCG. 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):450-9.
34. Longo-Mbenza B, Lukoki Luila E, M'Buyamba-Kabangu JR. Nutritional status, socio-economic status, heart rate, and blood pressure in African school children and adolescents. International Journal of Cardiology. 2007;121(2):171-7.
35. Agyemang C, Redekop W, Owusu-Dabo E, Bruijnzeels M. Blood pressure patterns in rural, semi-urban and urban children in the Ashanti region of Ghana, West Africa. BMC public health. 2005;5(1):114. PubMed PMID: doi:10.1186/1471-2458-5-114.
36. McDade TW. Early environments and the ecology of inflammation. Proc Natl Acad Sci U S A. 2012 Oct 16;109 Suppl 2:17281-8. PubMed PMID: 23045646. Pubmed Central PMCID: 3477398.
37. Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science. 2011 Apr 8;332(6026):243-7. PubMed PMID: 21436399. Pubmed Central PMCID: 3144160.
38. Hulman S, Kushner H, Katz S, Falkner B. Can cardiovascular risk be predicted by newborn, childhood, and adolescent body size? An examination of longitudinal data in urban African Americans. The Journal of pediatrics. 1998 Jan;132(1):90-7. PubMed PMID: 9470007. Epub 1998/02/21. eng.
39. Levitt NS, Steyn K, De Wet T, Morrell C, Edwards R, Ellison GT, et al. An inverse relation between blood pressure and birth weight among 5 year old children from Soweto, South Africa. Journal of epidemiology and community health. 1999 May;53(5):264-8. PubMed PMID: 10396531. Pubmed Central PMCID: 1756878. Epub 1999/07/09. eng.
40. Woelk G, Emanuel I, Weiss NS, Psaty BM. Birthweight and blood pressure among children in Harare, Zimbabwe. Archives of disease in childhood Fetal and neonatal edition. 1998 Sep;79(2):F119-22. PubMed PMID: 9828738. Pubmed Central PMCID: 1720834. Epub 1998/11/26. eng.
41. Margetts BM, Rowland MG, Foord FA, Cruddas AM, Cole TJ, Barker DJ. The relation of maternal weight to the blood pressures of Gambian children. Int J Epidemiol. 1991 Dec;20(4):938-43. PubMed PMID: 1800434. Epub 1991/12/01. eng.
42. Shulman CE, Dorman EK. Importance and prevention of malaria in pregnancy. Trans R Soc Trop Med Hyg. 2003 Jan-Feb;97(1):30-5. PubMed PMID: 12886801.
43. Williams RR, Hunt SC, Hasstedt SJ, Hopkins PN, Wu LL, Berry TD, et al. Are there interactions and relations between genetic and environmental factors predisposing to high blood pressure? Hypertension. 1991 Sep;18(3 Suppl):I29-37. PubMed PMID: 1889856.
44. Kidambi S, Ghosh S, Kotchen JM, Grim CE, Krishnaswami S, Kaldunski ML, et al. Non-replication study of a genome-wide association study for hypertension and blood pressure in African Americans. BMC medical genetics. 2012;13:27. PubMed PMID: 22494468. Pubmed Central PMCID: 3349540.
45. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. Science. 2010 Aug 13;329(5993):841-5. PubMed PMID: 20647424. Pubmed Central PMCID: 2980843. Epub 2010/07/22. eng.

46. Lule SA, Webb EL, Ndibazza J, Nampijja M, Muhangi L, Akello F, et al. Maternal recall of birthweight and birth size in Entebbe, Uganda. Tropical medicine & international health : TM & IH. 2012 Sep 20. PubMed PMID: 22994260. Pubmed Central PMCID: 3627817. Epub 2012/09/22. Eng.
47. Ndibazza J, Muhangi L, Akishule D, Kiggundu M, Ameke C, Oweka J, et al. Effects of deworming during pregnancy on maternal and perinatal outcomes in Entebbe, Uganda: a randomized controlled trial. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2010 Feb 15;50(4):531-40. PubMed PMID: 20067426. Pubmed Central PMCID: 2857962. Epub 2010/01/14. eng.
48. Ndibazza J, Mpairwe H, Webb EL, Mawa PA, Nampijja M, Muhangi L, 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):e50325. PubMed PMID: 23236367. Pubmed Central PMCID: 3517620. Epub 2012/12/14. eng.
49. Ezenwaka CE, Seales D, Surujlal R, Mathura RP. Glycated haemoglobin A1c measurement in stored whole blood sample is reliable for clinical use. The West Indian medical journal. 2009 Jan;58(1):17-20. PubMed PMID: 19565994. Epub 2009/07/02. eng.
50. Selvin E, Coresh J, Zhu H, Folsom A, Steffes MW. Measurement of HbA1c from stored whole blood samples in the Atherosclerosis Risk in Communities study. Journal of diabetes. 2010 Jun;2(2):118-24. PubMed PMID: 20923494. Pubmed Central PMCID: 2991637. Epub 2010/10/07. eng.
51. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004 Aug;114(2 Suppl 4th Report):555-76. PubMed PMID: 15286277. Epub 2004/08/03. eng.
52. 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 Oct;44(4):387-8. PubMed PMID: 15353515. Epub 2004/09/09. eng.
53. Wikoff WR, Kalisak E, Trauger S, Manchester M, Siuzdak G. Response and recovery in the plasma metabolome tracks the acute LCMV-induced immune response. Journal of proteome research. 2009 Jul;8(7):3578-87. PubMed PMID: 19496611. Pubmed Central PMCID: 3437991.
54. Tobin DM, Roca FJ, Oh SF, McFarland R, Vickery TW, Ray JP, et al. Host genotype-specific therapies can optimize the inflammatory response to mycobacterial infections. Cell. 2012 Feb 3;148(3):434-46. PubMed PMID: 22304914. Pubmed Central PMCID: 3433720.
55. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature. 2009 Jul 2;460(7251):103-7. PubMed PMID: 19494812. Pubmed Central PMCID: 2803086.
56. Tielens AG, van Grinsven KW, Henze K, van Hellemond JJ, Martin W. Acetate formation in the energy metabolism of parasitic helminths and protists. International journal for parasitology. 2010 Mar 15;40(4):387-97. PubMed PMID: 20085767.
57. Vinolo MA, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. Nutrients. 2011 Oct;3(10):858-76. PubMed PMID: 22254083. Pubmed Central PMCID: 3257741.
58. Royston P, Altman DG, Sauerbrei W. Dichotomizing continuous predictors in multiple regression: a bad idea. Statistics in medicine. 2006 Jan 15;25(1):127-41. PubMed PMID: 16217841. Epub 2005/10/12. eng.
59. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, et al. Genome-wide association study of blood pressure and hypertension. Nature genetics. 2009 Jun;41(6):677-87. PubMed PMID: 19430479. Pubmed Central PMCID: 2998712. Epub 2009/05/12. eng.
60. Taal HR, Verwoert GC, Demirkan A, Janssens AC, Rice K, Ehret G, et al. Genome-wide profiling of blood pressure in adults and children. Hypertension. 2012 Feb;59(2):241-7. PubMed PMID: 22203742. Pubmed Central PMCID: 3266432. Epub 2011/12/29. eng.

61. van El CG, Cornel MC, Borry P, Hastings RJ, Fellmann F, Hodgson SV, et al. Whole-genome sequencing in health care: recommendations of the European Society of Human Genetics. European journal of human genetics : EJHG. 2013 Jun;21(6):580-4. PubMed PMID: 23676617. Pubmed Central PMCID: 3658192. Epub 2013/05/17. eng.

14. Appendices

14.1. Appendix 1: Blood pressure levels charts for gender by Age and Height Percentile

Blood Pressure Levels for Boys by Age and Height Percentile

Age (Year)	BP Percentile ↓	Systolic BP (mmHg) ← Percentile of Height →							Diastolic BP (mmHg) ← 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

Blood Pressure Levels for Boys by Age and Height Percentile (Continued)

Age (Year)	BP Percentile ↓	Systolic BP (mmHg) ← Percentile of Height →							Diastolic BP (mmHg) ← Percentile of Height →						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
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

BP, blood pressure

* The 90th percentile is 1.28 SD, 95th percentile is 1.645 SD, and the 99th percentile is 2.326 SD over the mean.

For research purposes, the standard deviations in Appendix Table B–1 allow one to compute BP Z-scores and percentiles for boys with height percentiles given in Table 3 (i.e., 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%; 95% = 1.645) and then computed according to the methodology in steps 2–4 described in Appendix B. For children with height percentiles other than these, follow steps 1–4 as described in Appendix B.

Appendix 16

Blood Pressure Levels for Girls by Age and Height Percentile

Age (Year)	BP Percentile ↓	Systolic BP (mmHg) ← Percentile of Height →							Diastolic BP (mmHg) ← 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

Blood Pressure Levels for Girls by Age and Height Percentile (Continued)

Age (Year)	BP Percentile ↓	Systolic BP (mmHg)							Diastolic BP (mmHg)						
		← Percentile of Height →							← Percentile of Height →						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
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

BP, blood pressure

* The 90th percentile is 1.28 SD, 95th percentile is 1.645 SD, and the 99th percentile is 2.326 SD over the mean.

For research purposes, the standard deviations in Appendix Table B–1 allow one to compute BP Z-scores and percentiles for girls with height percentiles given in Table 4 (i.e., 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%; 95% = 1.645) and then computed according to the methodology in steps 2–4 described in Appendix B. For children with height percentiles other than these, follow steps 1–4 as described in Appendix B.