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LONGITUDINAL ANALYSIS OF GENETIC RISK FACTORS FOR CARDIOVASCULAR DISEASE: THE BIRTH TO TWENTY PLUS COHORT



Richard Junganiko Munthali

A THESIS

submitted to the School of Molecular and Cell Biology, Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of

Doctor of Philosophy

June 2017

Johannesburg, South Africa

Declaration

I, RICHARD JUNGANIKO MUNTHALI (student no.: 794575), am a student registered for the degree of Doctor of Philosophy (PhD) in the academic year 2016.

I hereby declare the following:

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Date: 5th June, 2017

Signature: 

Supervisors: Dr. Zané Lombard and Prof. Shane Norris

"The surge in obesity in this country is nothing short of a public health crisis that is threatening our children, our families, and our future…In fact, the health consequences are so severe that medical experts have warned that our children could be on track to live shorter lives than their parents." Michelle Obama, former First Lady of the United States (U.S. Department of Health and Human Services Press Release; January 28, 2010).

Abstract

Non-communicable diseases (NCDs) pose an increasing burden on public health and economic growth worldwide. The highest increase in prevalence and death rates of NCDs has been seen in low and middle-income countries (LMICs). World Health Organization (WHO) estimates that by 2030, NCDs will account for five times as many deaths as communicable diseases in LMICs and that there will be more than 2.16 billion overweight and 1.12 billion obese individuals in the world. It is also estimated that by 2020 NCDs will contribute 80 percent of the global burden of disease and the largest increase in NCD deaths will occur in Africa. Recent reports indicate that six of the ten leading natural causes of death in South Africa are NCDs.

There are few studies that have used longitudinal data to understand the effects of life-course childhood adiposity on future health risks and the early life factors responsible for variations in life-course childhood obesity. However, it is not known whether there is a genetic basis for the variability in BMI developmental patterns over time. Lack of comprehensive longitudinal and genetic association data for obesity have made it difficult to do such studies in an African setting. It is still not clear whether the same genetic variants associated with obesity in Europeans and other populations are also associated with these traits in African populations. Understanding the genetic contribution to obesity in the black South African population may help to come up with effective interventions to deal with this emerging epidemic in Africa.

The aim of this thesis was to better understand the contribution of genetics and explain the longitudinal genetic basis of childhood and adolescence obesity in black South African children. To deal with this, I firstly studied identification of distinct trajectories of BMI and then relate the established BMI trajectories to the future health risks of elevated blood pressure. Secondly, I explored the early life factors behind BMI trajectory membership, this would help to identify factors that may accelerate an individual's progression from a normal BMI trajectory pattern membership to the one characterized with elevated BMI. Then lastly, I looked at the additive genetic effect for BMI and determine whether genetic risk of obesity in early adulthood was mediated by early life rapid growth.

Results showed variation in BMI developmental patterns between boys and girls; three and four distinct sex-specific BMI trajectories were identified in boys and girls respectively. Children belonging to early onset overweight or obese BMI trajectories, characterized by elevated BMI, had an increased risk of elevated blood pressure in late adolescence, compared to their peers in the normal trajectories.

Rapid conditional relative weight gain in early life was associated with increased risk of belonging to a BMI trajectory characterized by consistent elevated BMI over time. Individuals in overweight or obese trajectories had a higher chance of being overweight or obese in early adulthood.

I found that a genetic risk score, based on known adult BMI Caucasian risk variants, showed significant longitudinal effects of genetic loci with BMI in childhood and adolescent and significant age-GRS interactions were observed. A higher genetic risk score predicted membership of early onset obese or overweight BMI trajectories. The genetic risk of obesity at 18 years of age was mediated by pre-adolescence and adolescence rapid weight gain.

The results from this thesis emphasize the importance of studying individual's BMI developmental patterns when studying development and progression of obesity. These findings also show that the information obtained from GWAS done in other populations can be equally relevant to African populations and this could be used in early identification of individuals at increased risk of obesity and other NCDs risk factors. Combing genetic risk score, BMI trajectories membership and weight status can be used to help improve the screening process of individuals to be targeted in coming up with targeted educational and behavior intervention programmes for obesity. These programmes should target individuals at risk at early stage in order to reduce the adverse health risk outcomes later in life.

Dedicated to my Lord and Savior, Jesus Christ

Let all that I am praise the Lord;

with my whole heart, I will praise his holy name.

Let all that I am praise the Lord;

may I never forget the good things he does for me.

Psalm 103: 1-2

In memory of my fallen grandparents, thank you for your love, wisdom, encouragement and the wonderful memories. I will always love you.

Acknowledgements

My sincere gratitude goes to my principal supervisor, Dr. Zané Lombard for giving me a chance to be part of her interdisciplinary research group. Thanks to both of my supervisors, Dr. Zané Lombard and Prof. Shane Norris, for their excellent support and advice, not just on my research, but also on my professional development and on the writing of my thesis. Through their expertise and connections, I was in the lucky position to learn from and work with leading researchers in epidemiology, bioinformatics as well as genetics. Thank you for the many opportunities you have given me. I also would like to thank my PhD advisor Dr Debbie de Assis Rosa. To Prof. Nicola Mulder and Dr. Emile Chimusa Rugamika, thank you for introducing me to the field of bioinformatics and computational biology.

I would like to thank Dr. Ken Ong and Dr. Felix Day for their valuable input and encouraging guidance rendered tirelessly throughout the period when I was a visiting student at the University of Cambridge. Many thanks should go to Prof. Nick Wareham and Dr. Rebecca Stratford, researchers, postdocs, students and staff of the MRC Epidemiology Unit at University of Cambridge who made my stay in Cambridge a fruitful one.

To students, postdocs, researchers and staff of Sydney Brenner Institute for Molecular Bioscience and MRC/Wits Developmental Pathways for Health Research Unit, thank you very much for the encouragement and help you rendered to me during my PhD.

I wish to thank the Bt20+ participants for taking part in the study and the Bt20+ team for their relentless support throughout the study.

I would like to thank the following funding bodies that contributed in one way or another during the course of my PhD studies; National Research Council Funding of South Africa, African Institute for Mathematical Sciences - Next Einstein Initiative (AIMS-NEI), University of the Witwatersrand, and the University of Cambridge and University of the Witwatersrand PhD partnering RCUK Newton Fund.

Finally, I want to thank my family, my parents Peter and Mary, for all their love and support. I love all of you. To all those individuals who have played a role to my long academic journey, I say stay blessed.

Abbreviations

BLRT	Bootstrap Likelihood Ratio Test
BMI	Body mass index
Bt20+	Birth to Twenty Plus Cohort Study
cAMP	responsive element binding protein 3 regulatory factor
CI	Confidence interval
COMT	catechol-O-methyltransferase
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
df	Degrees of freedom
DNA	Deoxyribonucleic acid
DOHaD	Developmental origins of health and disease
EM	Expectation maximization
FTO	fat mass and obesity-associated protein;
GRS	genetic risk score
GWAS	Genome-wide association study
HICs	High-income countries
HTR2A	5-hydroxytryptamine receptor 2A
HWE	Hardy-Weinberg equilibrium
IOTF	International Obesity Task Force
LCGA	Latent Class Growth Analysis
LCGMM	Latent Class Growth Mixture Modeling
LD	Linkage disequilibrium
LMICs	Low-middle-income countries
LMR-LRT	Lo, Mendell and Rubin Likelihood Ratio Test
MAF	Minor allele frequency
MAR	Missing at random
MC4R	melanocortin 4 receptor;
ML	Maximum likelihood
NCDs	Non-communicable diseases
OR	Odds ratio
QC	Quality control
SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error
SES	Socio-economic status
SLC6A3A	Solute Carrier Family 6 (Neurotransmitter Transporter, Dopamine), Member 3
SNP	Single nucleotide polymorphism
T2D	Type 2 diabetes
WHO	World Health Organization

Publications and Presentations

Publications

I. **Munthali, R.J**., Kagura, J., Lombard, Z. and Norris, S.A., 2016. Childhood adiposity trajectories are associated with late adolescent blood pressure: birth to twenty plus cohort. ***BMC Public Health***, 16(1), p.665.

Student's contribution to the paper

The student carried out the initial conceptualization and design of the study, led the study, conducted the initial analyses and interpreted the data, drafted the initial manuscript, and approved the final manuscript as submitted.

II. **Munthali, R.J**., Kagura, J., Lombard, Z. and Norris, S.A., 2016. Early life factors are associated with childhood adiposity trajectories and adult obesity: the Birth to Twenty Plus Cohort (Online Ahead of Print: ***Childhood Obesity, May 2017***); DOI: 10.1089/chi.2016.0310

Student's contribution to the paper

The student carried out the initial conceptualization and design of the study, led the study, conducted the initial analyses and interpreted the data, drafted the initial manuscript, and approved the final manuscript as submitted.

III. **Richard J Munthali**, Venesa Pillay, Juliana Kagura, Liesl M Hendry, Shane A Norris, Ken K Ong, Felix R Day, Zané Lombard, 2017. Genetic risk score for adult body mass index associations with childhood and adolescent weight gain in an African population (under review; ***Genes & Nutrition)***

Student's contribution to the paper

Conception and design of the study, analyzed and interpreted data, wrote a first draft of the manuscript and approved final version to be published.

Poster Presentations:

I. **Richard J Munthali**, Venesa Pillay, Juliana Kagura, Liesl M Hendry, Shane A Norris, Ken K Ong, Felix R Day, Zané Lombard, 2016. ***Association between genetic risk score and body mass index in an African population: mediating roles of childhood and adolescent weight gain.*** Presented at the Ninth H3Africa Consortium Meeting, 28-31 October 2016, Le Meridien Ill Maurice, Mauritius.

II. **Richard J Munthali**, Venesa Pillay, Juliana Kagura, Liesl M Hendry, Shane A Norris, Ken K Ong, Felix R Day, Zané Lombard, 2016. ***Association between genetic risk score and body mass index in an African population: mediating roles of childhood and adolescent weight gain***. Presented at the 2016 Molecular Bioscience Research Thrust (MBRT) Research Day, 8 November 2016, University of the Witwatersrand, South Africa

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Part 1

Background, Literature Review and Methodology

Chapter 1: Background and Literature Review

1.1 Rationale

Recently, non-communicable diseases (NCDs), such as cardiovascular diseases (CVD), diabetes, chronic obstructive pulmonary diseases (COPD) and cancers, are leading causes of mortality and morbidity worldwide (Terzic et al., 2011). Many common risk factors, such as smoking, elevated blood pressure (BP), overweight and/or obesity, harmful alcohol consumption, unhealthy diet, physical inactivity, blood cholesterol and glucose levels, have been associated with NCDs mortality and morbidity (Perk et al., 2012). The highest increase in rates of NCDs have been seen in low and middle-income countries (LMICs) placing an increasing impact of health burden in these populations, 80% of NCD deaths (29 million) occurring in LMICs (Alwan, 2011a). It is estimated that by 2020 NCDs will contribute 80 percent of the global burden of disease and the largest increase in NCD deaths will occur in Africa (Abegunde et al., 2007; Alwan, 2011b; Geneau et al., 2010; Mathers et al., 2006; WHO, 2013a). Furthermore WHO estimates that in African nations, deaths from NCDs will exceed the combined deaths of communicable and nutritional diseases, and maternal and paternal deaths as the most common causes of death by 2030 (Kelly et al., 2008). The global NCD burden is estimated to increase by 17% by 2025, and in the African region by 27% (WHO, 2013a). Obesity and hypertension have reached epidemic levels in high-income countries and are emerging epidemics in LMICs (Kelly et al., 2008; Ng et al., 2014). For this reason, NCDs and NCD risk factors prevention pose high public health significance worldwide.

Even childhood obesity has been on the rise in LMICs where the prevalence rates have increased at alarming rates in the past three decades. It is estimated that more than 43 million under the age of five children are overweight globally, 35 million of them living in LMICs (De Onis et al., 2010; WHO, 2016)**.** Africa registered the fastest overweight and obesity growth rate in the world between 1990 and 2010. The number of overweight or obese children more than doubled in this period (De Onis et al., 2000; De Onis et al., 2010). Children who are overweight or obese are likely to stay obese into adulthood and more likely to develop NCDs like diabetes and cardiovascular diseases earlier (Rolland-Cachera et al., 1987; Taveras et al., 2011). Therefore, the likelihood of future increase of NCDs burden in the LMICs if no intervention is implemented is certain. This calls for the need to put up strategies that would slow or reverse this trend in LMICs. The WHO emphasizes that timely, appropriate and valid surveillance of NCDs and their risk factors are needed by governments to

support the planning, implementation and evaluation of public health prevention and intervention strategies, which are crucial to address the growing burden of NCDs in low and middle income countries, such as South Africa (WHO, 2007). Despite all the threats NCDs pose to health status of the population and health systems in LMICs, research resources allocated to NCDs in LMICs are trivial which results in a paucity of data on NCDs in these countries (Beaglehole et al., 2008).

Obesity and high blood pressure are the most common metabolic risk factors of NCDs. Both obesity and raised blood pressure are multifactorial complex traits with both environmental and genetic risk factors modifying disease susceptibility, but genetic factors are not well understood (Herrera et al., 2011). Through genome wide analysis studies (GWAS) and target gene approaches, many genes have been associated with obesity in European populations (Locke et al., 2015). Despite the success of genetic studies in identifying genetic variants associated with obesity in European populations, there has been under-representation of such studies in African populations (Adeyemo et al., 2010; Yako et al., 2015). There is still much research to be done to understand heritability and progressing of obesity and hypertension in African populations. It is still not clear whether the same genetic variants associated with obesity in Europeans and other populations are also associated with these traits in African populations. There is still lack of genetic association data for obesity in African populations (Yako et al., 2015). Understanding the genetic contribution to obesity and/or hypertension in the black South Africans may help to come up with effective interventions to deal with this soaring epidemic in Africa.

The association between genetic variants and phenotype such as body mass index (BMI) may vary across the life-course (Rzehak et al., 2010). Most genetic studies looking for associations between genetic variants and disease risk make use of cross-sectional phenotype data (Chiu et al., 2016; Kerner, North, et al., 2009; Locke et al., 2015; Smith et al., 2010; Ware et al., 2015; Wu et al., 2014; Yako et al., 2015). Though such research has reported many genetic risk factors, some associations may have been missed depending on the timing when the phenotype used was collected. There are few studies (both in African and non-African cohorts) that have used longitudinal associations to describe the genetic effect of obesity across childhood through to young adulthood (Fulford et al., 2015; Hung et al., 2015; Monnereau et al., 2016; Smith et al., 2015; Zhu et al., 2014). Lack of comprehensive longitudinal phenotype and genetic data have made it difficult to do such studies in an African setting. The use of longitudinal data analysis approaches has hardly been explored in any genetic study on obesity in the African populations before.

This study takes advantage of group-based trajectory modeling to redefine longitudinal body mass index (BMI) phenotypes for longitudinal genetic association. This method takes heterogeneous longitudinal data and analyses it into homogeneous subgroups. A group based modeling called Latent Class Growth Mixture Modeling (LCGMM) was employed (Jones et al., 2001; Kerner & Muthén, 2009), see Section 2.4. LCGMM is a less explored statistical tool in genetics studies and still new to epidemiological and biological research. It predicts both number of trajectories and trajectory membership. The Bayesian posterior probabilities of belonging to a given trajectory are used as phenotype in association analyses. A posterior probability is the probability of assigning individuals to groups based on the given data; it is based on application of the Bayes' theorem (Hoekstra, 2013; Koch, 1990; Nagin et al., 2010). The BMI trajectories are used as proxy phenotype in traditional GWAS and other association methods. The other approach used in this study is genetic risk score, by summing up the number of risk alleles for disease prediction. The genetic risk scores are not affected by the background noise such as age, ethnicity and environment (Cooke Bailey et al., 2016; Monnereau et al., 2016; Smith et al., 2015). The risk score is used as an exposure in regressions and other longitudinal data analysis tools. In doing so we reduce the computational power and model complexity that comes with analyzing longitudinal data.

The main purpose of this study is to use longitudinal data analysis methods to characterize variations in BMI developmental patterns and explore the influence of genetic variants on obesity in the black South African population. It is unclear, whether the gene(s) have the same genetic effect on BMI across all ages or there exist age specific genetic effects. Knowing the genes that are associated with how obesity develops over time can provide the opportunity for preventive measures before disease onset and can potentially be used to screen young individuals (Smith et al., 2010).

Using the longitudinal data of Birth to Twenty Plus cohort (Bt20+), I perform a longitudinal study to characterize variation in the BMI developmental patterns then do genetic association focusing on genetic markers associated with adult BMI measures in Europeans, using genotypes assayed with the Metabochip DNA arrays (Illumina, USA) (Voight et al., 2012), to find out if these markers are similarly relevant important in genetic obesity risk in Africans. This study will help to understand the genetics effects and progression of obesity in black South African population.

1.2 Aims and Objectives

1.2.1 Overall aim

The overall aim of this thesis is to enhance understanding and gain new insights into weight trajectories leading to obesity and elevated blood pressure risks, and genetic risk factors of obesity from childhood to late adolescence. These insights would possibly help in developing new means of effective prevention and treatment strategies of NCDs in the future.

1.2.2 Specific objectives

In this study, I looked at the following objectives:

1. Investigate variation in body mass index developmental patterns and future health risks of elevated blood pressure.
    a) Identify distinct BMI trajectories from 5 to 18 years.
    b) Assess whether the identified BMI trajectories differ between boys and girls.
    c) Find the prevalence of elevated blood pressure at 18 years.
    d) Explore associations between the distinct adiposity trajectories' membership and elevated blood pressure in late adolescence.
2. Examine the early life growth factors, such as relative conditional weight gain and birth weight, associated with particular BMI trajectory membership, and explore the associations between the distinct adiposity trajectories' membership and overweight risk in young adulthood.
    a) Assess the association between early-life growth factors and BMI trajectories from ages 5 to 18 years.
    b) Find the prevalence of overweight or obesity in young adulthood.
    c) Explore association between BMI trajectory membership and risk of overweight or obesity in young adulthood at the age of 23 years.
3. Explore the genetic basis of obesity by investigating genetic risk of longitudinal BMI measures.
    a) To combine a collection of the extracted genetic alleles associated with adult BMI into a single number, as a genetic risk score, which can be used for genetic risk assessment.

b) To test association between weighted multi-SNPs genetic risk score based on adult BMI associated variants with longitudinal measures of BMI from birth to 18 years old of age.
c) Examine the association between the genetic risk score and the BMI trajectories membership reported in Objective 1.
d) Explore whether genetic risk of obesity at 18 years old of age was mediated by conditional relative weight gain at different growth periods.

1.2.3 Study hypotheses

The following null hypotheses were tested in this study:

i. There are no different sex-specific distinct adiposity growth patterns among South African children and variations in growth patterns have no effect on future health risks.
ii. Birth weight, early life weight gain and genetics are not predictors of BMI trajectory membership.
iii. Polygenic genetic risk scores have no effect on longitudinal BMI through infancy and adolescence.
iv. Both childhood and adolescent growth have no mediation role on genetic influence on adult BMI.

1.3 Conceptual Model and Theoretical Framework

The overview framework of the thesis was constructed from available literature. A life-course perspective was employed to conceptualize weight status, early life factors for variation in weight status, genetic risk factors and health outcomes in this thesis. The life-course approach entails that the outcomes of a developmental trajectory from childhood progresses into adulthood. Early childhood experiences influence adulthood life later in life (Howe et al., 2015). Different research approaches have shown that overweight and obesity during childhood can shape the health in adulthood in a population. Little has been done to explore how individuals' weight change vary over time, how genetics play a role in these changes, how these weight changes mediate genetic associations and how particular trajectories of weight change individuals health in future.

The heterogeneity in weight change can be captured using LCGMM, which captures the changes in BMI over time in a population. This is useful for identifying homogeneous subpopulations within the larger heterogeneous population and for the identification of the groups of interest such as the group at risk of the outcome of interest (Jung et al., 2008). The framework includes predictors and

moderators of BMI class membership, and later life health risk outcomes based on BMI trajectory membership, Figure 1-1. Moderating variable is that affects the direction and/or strength of the relation between an exposure or predictor variable and an outcome variable. Genetic association analysis was done after establishing the BMI trajectories. The proposed conceptual model is based on different international studies on American children (Weiss, 2012), however Weiss did not explore whether genetic variants can predict the BMI trajectory membership and the mediation roles of childhood and adolescent growth in the genetic associations. This study goes one step further in African population by including genetic analyses.

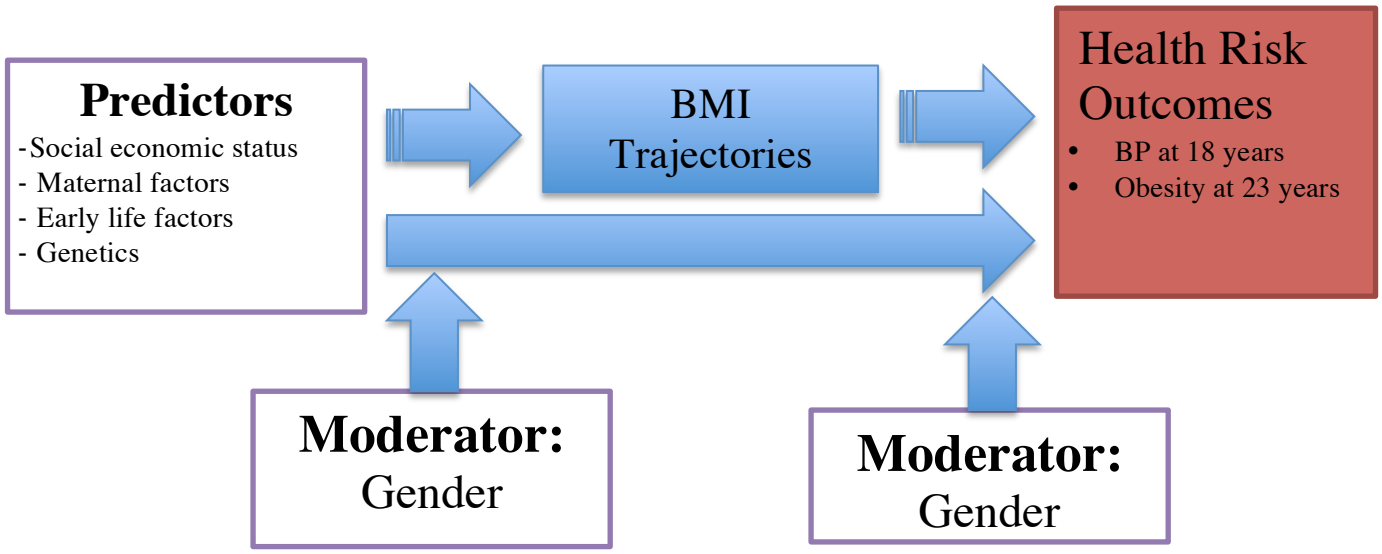

Figure 1-1: Conceptual Diagram; BMI variations over time, early life factors and later life health risks. Modified: adapted from (Weiss, 2012).

1.4 Thesis Overview and Structure

This thesis has three empirical chapters. The first empirical chapter presents the first part of the study investigating variation in BMI developmental patterns and future health risks of elevated blood pressure. Then, the second empirical chapter follows up on the results presented in the first by investigating the early life growth factors associated with particular BMI trajectory membership and explore the associated risk of overweight or obesity in young adulthood. Finally, the third empirical chapter presents a study exploring the genetic basis of NCDs by investigating genetic risk of

longitudinal BMI measures as indicator of obesity, which is one of the major metabolic risk factors of NCDs.

1.5 Literature Review

1.5.1 Non-Communicable Diseases (NCDs)

Non-communicable diseases (NCDs), also known as chronic diseases, are diseases which are not transmissible or caused by injury and include mainly cardiovascular diseases (CVD), cancers, musculoskeletal disorders, chronic respiratory disease and diabetes (Abegunde et al., 2007; Yach et al., 2004). The problem of non-communicable diseases remains an area of public health focus globally, in 2012, 38 (68%) of 56 million global deaths were due to NCDs (Mozaffarian et al., 2016). WHO estimates that by 2030, NCDs will account for five times as many deaths as communicable diseases in LMICs (Mathers et al., 2006). There is growing concern of an epidemic of NCDs, which calls for finding prevention mechanisms to stop this emerging epidemic in these countries. This comes at a time when much of the funding in LMICs is channeled towards the prevention of nutritional disorders and infectious diseases such as Tuberculosis and human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) (Hofman, 2014; Maher et al., 2012; Marquez et al., 2013). This leaves prevention and treatment of NCDs being relatively sparsely funded.

Evidence shows that the burden of NCDs in South Africa has increased over the past two decades (Puoane et al., 2012). Deaths from NCDs, including CVDs contribute for 43% of total adult deaths in South Africa, of which 18% are due to CVDs (WHO, 2014). A recent report released by Statistics South Africa indicates that six of the top ten natural causes of death among South Africans in 2015 were NCDs. Diabetes was the second leading natural cause of deaths accounting for 5.4% of the deaths just behind Tuberculosis (TB), which accounted for 7.2% of deaths (STATSSA, 2017). Diabetes rates of mortality climbed from fifth position in 2013 to second position in 2015.

CVDs are the most common type of NCDs. Over three quarters of CVD deaths occur in LMICs (Mozaffarian et al., 2016). In 2013, nearly one million deaths were caused by CVDs in Sub Saharan Africa (SSA) representing 5.5% of global CVD deaths (Mensah et al., 2015), A systematic review by Mensah and colleagues reported that though CVDs were not among the leading cause of death in SSA, there was a significant increase in number (80%) of deaths from CVD in 2013 compared to 1990 (Mensah et al., 2015). In South Africa at least 210 people die from heart disease every day (Zuhlke, 2016).

The NCDs and NCD risk factors impact not only the health but also social life, the environment and economy of countries (CU, 2016). Since most NCDs are chronic conditions, patients need to adjust their lifestyle such as not smoking or not drinking alcohol. Due to physical, social and decline health, NCDs patients or their family caregivers increase risk of becoming depressed which in turn increases the risk of NCDs (Hare et al., 2013). NCDs also have environmental effects through different pathways. NCDs and their risk factors also pose economic challenges, as they stretch the financial budgets for governments and derail health systems operations. In 2010 the global cost for CVD was US$ 863 billion and this was estimated to rise by 22% by 2030 (Bloom et al., 2012) and in South Africa, it was estimated that US$1.88 billion gross domestic product was lost due to diabetes, stroke and coronary heart attack between 2006 and 2015 (Hofman, 2014). Understanding the epidemiology of NCDs is important more especially in SSA which has seen epidemiological transition with the rates of NCDs increasing (Bain et al., 2013; Maredza et al., 2011). It is therefore important that causes of NCDs and concerns on their impacts should be addressed. This would also help to formulate regional and national health policies within the SSA region. Finding ways of reducing obesity prevalence in adults and preventing overweight at an early age is very important in public health (Mei et al., 2012).

1.5.2 Non-Communicable Disease Risk Factors

Most NCDs share common risk factors, which are often categorized as modifiable, largely due to life style, and non-modifiable NCD risk factors (Causer, 2014; Franks et al., 2010; Lim et al., 2013; Musa et al., 2016; Sliwa et al., 2008; Tibazarwa et al., 2009). Modifiable factors are further categorized as behavioral such as current tobacco smoking, harmful alcohol consumption, unhealthy diet (e.g. high salt intake), sleeping patterns and physical inactivity or biological such as overweight and/or obesity, high cholesterol levels, high blood pressure and raised blood glucose levels, see Figure 1-2, (Aryal et al., 2015; Hoy, 2013; Hunter et al., 2013; Li et al., 2013). Targeting these modifiable risk factors can prevent most of the NCDs (Kontis et al., 2014). On the other hand, male gender, ethnicity, family history and genetics are examples of non-modifiable risk factors (Lloyd-Jones et al., 2010; Watkins, 2004). Apart from being risk factors for CVDs, the non-modifiable risk factors also influence the risk of the traditional modifiable risk factors.

Recent studies show that other emerging CVD risk factors, such as increased levels homocysteine, have an influence on the development of CVD (Akhabue et al., 2014; Balagopal et al., 2011). Other social cultural factors such as literacy levels, psychosocial stress, access to health care and socio-

economic status also affect risk for CVD and other NCDs (Havranek et al., 2015; Henry-Okafor, 2009; Kreatsoulas et al., 2010).

1.5.2.1 Modifiable risk factors

It should be stated that modifiable effects are as a result of both genetic and non-genetics backgrounds. For example, genes affect many of the factors contributing to adult BMI, such as endocrine and metabolic diseases. Most of these modifiable risk factors are as a result of the complex interaction between an individual's genetic make-up and the environmental factors that he or she is exposed to.

High blood pressure

Hypertension is a medical condition referring to elevated blood pressure in arteries. Blood pressure is usually expressed in terms of the systolic pressure (maximum during one heart beat) pressure over diastolic pressure (minimum in between two heart beats). According to Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (Chobanian, 2003), the following categories of blood pressure have been established for adults 18 years of age and older as shown in Table 1-1. These cut-offs were utilized in this study.

Table 1-1: Blood pressure measures

Classification	**Systolic Blood Pressure**		**Diastolic Blood Pressure**
Low	<90	or	< 60
Normal	<120	and	<80
Prehypertension	120-139	or	80-90
High: Stage 1 Hypertension	140-159	or	90-99
High: Stage 2 hypertension	≥160	or	≥100

Hypertension is one of the major risk factors for NCDs. High blood pressure stresses the walls of the blood vessels, weakening them and leading to damage of the blood vessels. This is mainly due to thickening of blood vessels since more blood will have to flow through a reduced volume. Hypertension is the single most important risk factor for stroke (WHF, 2016b). About 50% of ischaemic strokes are due to hypertension. One billion people are hypertensive worldwide (WHO, 2013b) and one in three South African adults (33.7%) have high blood pressure (WHF, 2016b), and are at high risk of CVDs and other NCDs

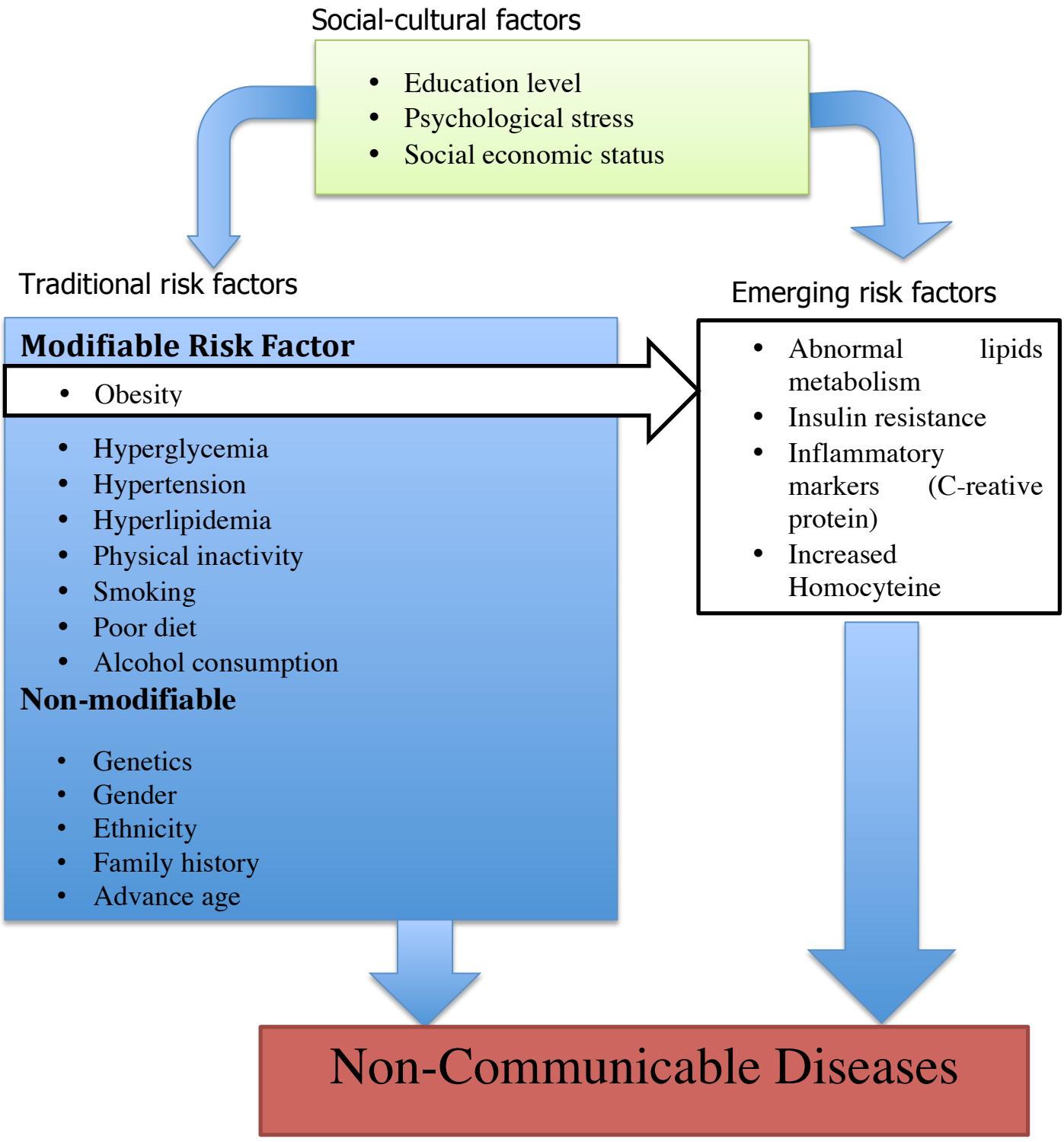

Figure 1-2: Factors contributing to non-communicable diseases.

Source: adapted from (Darnton-Hill et al., 2004)

Smoking

According to the recent WHO report, tobacco use kills about six million people per year. In South Africa, 32.1% of adult men and 7.4 % of adult women are smokers (WHO, 2015). Tobacco contains harmful chemicals and influences the development of plaque depositions. Plaque can build up in arteries, narrowing them, hence limiting the flow of oxygen rich blood in the body. Tobacco is associated with different other CVD and NCDs risk factors such as obesity, hypertension and also increases the CRP levels.

Hyperglycemia

Hyperglycemia (raised blood sugar) narrows the blood vessels resulting in damage of the blood vessels. Diabetic individuals are two to four times likely to develop heart disease than non-diabetics (WHF, 2016a). In 2009, it was estimated that 9% of people aged 30 years and older were diabetic in South Africa (Bertram et al., 2013). In 2015, diabetes was the leading underlying natural cause of death responsible for 7% of total deaths in South African women (STATSSA, 2017).

Physical inactivity

Physical activity is good for the heart and circulatory system. Physical inactivity or sedentary life is associated with high blood pressure, high cholesterol levels and likelihood of becoming overweight or obese; all of these are risk factors for NCDs. When combined with a healthy diet, exercise can also help reduce CVD risk (Buttar et al., 2005) .

High cholesterol levels

Cholesterol is a type of fat found in the blood. High cholesterol increases the risk of CVD by narrowing the blood vessels, which increases the risk of developing a blood clot. At least eight million people have high cholesterol levels in South Africa (Sifingo, 2016).

Alcohol

Excessive alcohol intake is associated with increased risk of cholesterol and blood pressure levels, and contribute to weight gain (Nelson et al., 2013). On the flip side, extensive research has shown that moderate alcohol intake is associated with some health benefits such as lower levels of cardiovascular disease, diabetes and hypertension (O'Keefe et al., 2007).

Obesity and poor diet

An unhealthy diet may lead to high cholesterol levels and high blood pressure. There has been an increase in total energy, fat and protein from animals and decrease in total carbohydrates taken during urbanization in LMICs (Popkin, 2002). High intake of processed meat such as bacon, sausages and ham increases the risk of CVDs and is associated with increased mortality rate in American and European studies. High salt intake from processed foods and other sources also increases the risk of heart disease and stroke (He et al., 2014; Rohrmann et al., 2013). South Africa has been undergoing nutritional and economic transitions hence seen a boom in fast foods and sugar sweetened beverages industries (Steyn et al., 2014; Tathiah et al., 2013). Fast foods are rich in fat which accumulates inside the arteries, this increases the risk of obesity, hypertension and hyperlipidemia (Hurt et al., 2010).

Obesity is increasingly becoming a public health problem issue with increase in incidences in obesity across the growth spectrum from childhood to old age, more especially in LICMs. It is estimated that by 2030 there will be more than 2.16 billion overweight and 1.12 billion obese individuals in the world (Alwan, 2011b). Though some high-income countries are registering a decrease in the prevalence of overweight and obesity among infants, children and adolescents, there is an increase in incidents of obesity and overweight in LMICs (De Onis et al., 2010; UNICEF, 2012). 12 million children are obese or overweight in Africa (WHO, 2016). South Africa is not spared from this emerging global problem with 40% and 70% of men and women being either overweight or obese, respectively while one in four girls and one in five boys between the ages of 2 and 14 years are overweight or obese in South Africa (Ng et al., 2014).

Both adulthood and childhood obesity are associated with increased cardiovascular risk and other CVDs. Obese adults who were obese in childhood have increased risks of other CVD and other NCDs risk factors such as type 2 diabetes, hypertension and dyslipidemia, compared to those who were not (Juonala et al., 2011). So being overweight or obese increases your risk of developing diabetes and high blood pressure, both of which are major risk factors for CVD (Chesi et al., 2015; Dhana et al., 2016; Kemp et al., 2012).

Variations in the prevalence of obesity across Africa have been reported before, ranging from 5.3 % in Uganda to 30 % in Nigeria and 45.7% in South Africa (Ng et al., 2014). These variations could be attributed to differences in economic growth, nutritional transition, environment and variations in genetic structure within African populations among others (Yako et al., 2015). South Africa like

other countries in SSA is undergoing a health transition, characterized by a decrease in infectious diseases and an increase in chronic diseases.

Since the dawn of democracy of democracy in 1994, South Africa has seen fast economic growth in the past two decades both in urban and rural areas. This economic growth comes with nutritional transition, which has coincided with the emergence of fast foods outlets and increase in sugar-sweetened drinks, change in eating behaviors, sedentary behaviors and lower physical activities (Iversen et al., 2011). This has led to the increase in the prevalence of cardiometabolic disease risk factors such as obesity and overweight. The cultural perception of a big body or a chubby baby as a healthy/ideal body is also attributing to the increase in these obesity incidences more especially in the black South African population compared to other populations (Kruger et al., 2014; Pienaar, 2015).

BMI is the simplest measure of obesity. According to the International Obesity Task Force (IOTF), the following BMI cut-offs are used to describe weight status (BMI: kgm^{-2}), underweight: less than 18.5, normal weight: 18.5 to 24.9, overweight: 25-29.9, obesity: 30 and above. In this study we used the IOTF BMI cut-offs (Cole et al., 2012).

1.5.2.2 Non-modifiable risk factors

Age

NCDs are most common in people over 50 years of age and the risk of developing them increases, as one gets older. As we age, we all develop higher risk for NCDs risk factors such as high blood pressure and cardiovascular disease since blood vessels lose flexibility with age. Though the symptoms of most NCDs emerge in adult life, early physiological and metabolic indicators can be detected long before that, even in childhood. Some risks are programmed during foetal life and only appear later in life (Prescott, 2015).

Gender

Men are more likely to develop NCDs at an earlier age than women while most women die of NCDs in the United States of America (USA), this is argued though, knowing that more women live beyond 60 years than men (Mosca et al., 2011). Since signs of NCDs develop later in women, they are at a higher risk of developing NCDs later in life than men (Norton, 2016).

Ethnicity

In the United Kingdom (UK), CVD is more common in people of South Asian and African or Caribbean background while in the USA, African-American men and women have higher prevalence

rates of CVDs and other NCDs than among white men and women (Go et al., 2013; Lip et al., 2007; O'Keefe et al., 2007). This is because people from these backgrounds are more likely to have other risk factors for NCDs, such as high blood pressure or T2D. In South Africa, NCDs used to be thought to be a problem for the white population, but recent studies have shown that there is a worsening progression of NCDs and NCDs risk factors in the black population (Hamer et al., 2015).

Figure 1-3 shows the progression of obesity via hypertension and other pathways to NCDs based on traditional risk factors as discussed above. With an increase in childhood obesity cases and tracking of obesity from childhood to adulthood, several risks factors for NCDs also start to emerge early in life hence an increase in risk for NCDs from childhood (Balagopal, 2006; Juonala et al., 2011). Taking a life-course approach to study NCDs and the risk factors would help to understand the onset and progression of these risk factors. This study is based on the bold big arrows pathway shown in Figure 1-3.

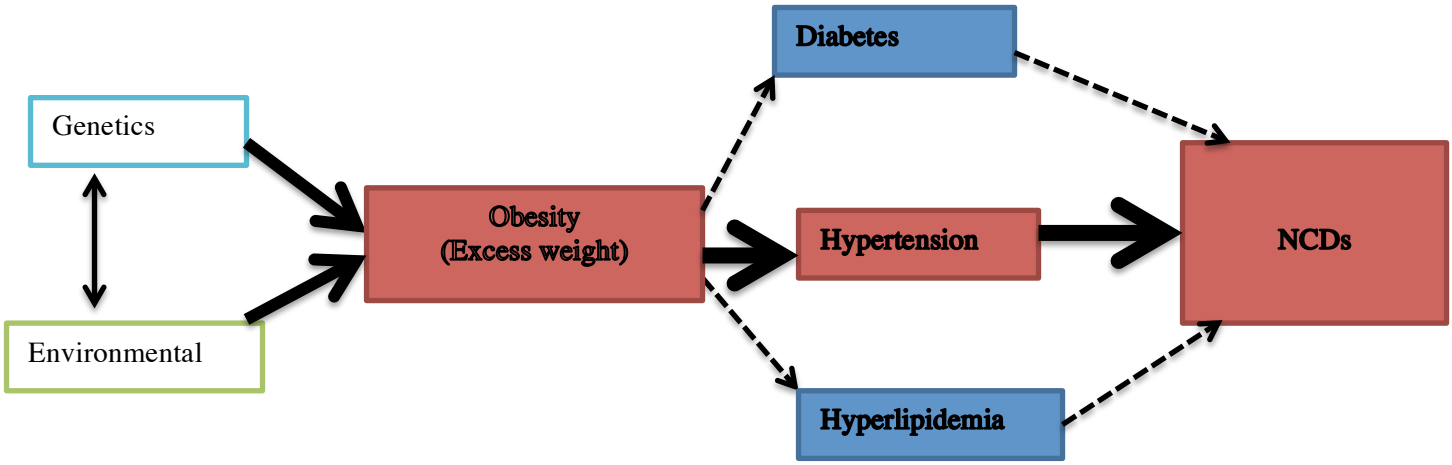

Figure 1-3: Schematic representation of progression of obesity to hypertension and NCDs.

1.5.3 Genetics

1.5.3.1 Key concepts

Selected basic genetic concepts and relevant for this thesis are explained in Table 1-2 and some of the concepts are further illustrated in Figure 1-4. The definitions are adapted from Burmeister (1999) and Burton et al. (2005) (Burmeister, 1999; Burton et al., 2005).

Table 1-2: Key concepts related to genetic studies.

Concept	Description
DNA	Deoxyribonucleic acid; the genetic information needed for the development and functioning of a living organism. The information in DNA is stored as a code made up of four chemical bases: adenine (A), cytosine (C), guanine (G) or thymine (T)). These bases pair each other, A with T and C with G, to form units called base pairs.
Gene	A DNA sequence that has the hereditary information. Genes act as instructions to make molecules called proteins. The estimated number of genes in humans is between 19,000 to 20,000 genes (Ezkurdia et al., 2014)
Genome	The complete DNA sequence; stored on one or more chromosomes. A human genome is made up of 23 chromosome pairs with a total of about 3 billion DNA base pairs.
Chromosome	An organized structure of DNA and protein. Diploid organisms (like humans) have two pairs of each chromosome with one member of each pair inherited from each parent. Humans have 22 pairs of autosomes and one pair of sex chromosomes. Thus, diploid organisms have two copies of each gene.
Locus	A position on the chromosome at which a particular gene is located
Allele	A variant form of a gene or a marker that is located at a specific position on a specific chromosome.
Genotype	The allelic makeup of an individual or a genetic makeup of an individual.
Homozygous	An individual with two identical copies of an allele.
Heterozygous	An individual with two different alleles.
Haplotype	A combination of alleles at multiple linked loci that are transmitted together.
MAF	Minor allele frequency; the frequency at which the less common allele occurs in a population.
HWE	Hardy-Weinberg equilibrium; the equilibrium between both the allele and

	the genotype frequencies in a population. The equilibrium may not hold if specific forces, e.g. mutation and non-random mating, genotyping errors and population stratification, have altered the allele frequencies.
LD	Linkage disequilibrium; a non-random statistical association of alleles at two or more loci. LD increases false positives since we observe significant associations that are not independently associated with a phenotype but associations between alleles or loci.
Population stratification	Differences in genotype frequencies between populations due to systematic ancestral differences.
SNP	A single nucleotide polymorphism; a variation at a single position in a DNA sequence (A, T, G or C) between members of a species or between paired chromosomes in an individual. A common SNP is a variant with MAF ≥ 1% and those with MAF < 1% are regarded as rare SNPs.
GWAS	A genome-wide association study; A large number of SNPs genotyped throughout the genome are tested for an association with a phenotypic trait of interest.
GRS	Genetic risk score, number based on variation in number of effect alleles in SNPs and their associated weights.
Imputation	It is the statistical inference of unobserved genotypes.
Fine mapping	The process of adding SNPs or other genetic variants which were not included in the original genotyping platform for a gene or the locus that has been replicated to be associated with a trait or disease (Farh et al., 2015).
Candidate gene	It is a genetic association method that focuses on genetic effect of pre-specified genes of interest on phenotypes.
Mutation	The changing of DNA sequence of a gene, which may result to alter the genetic message carried by that gene.

Concepts modified from www.genome.gov/Glossary or https://ghr.nlm.nih.gov/primer/basics/ otherwise stated

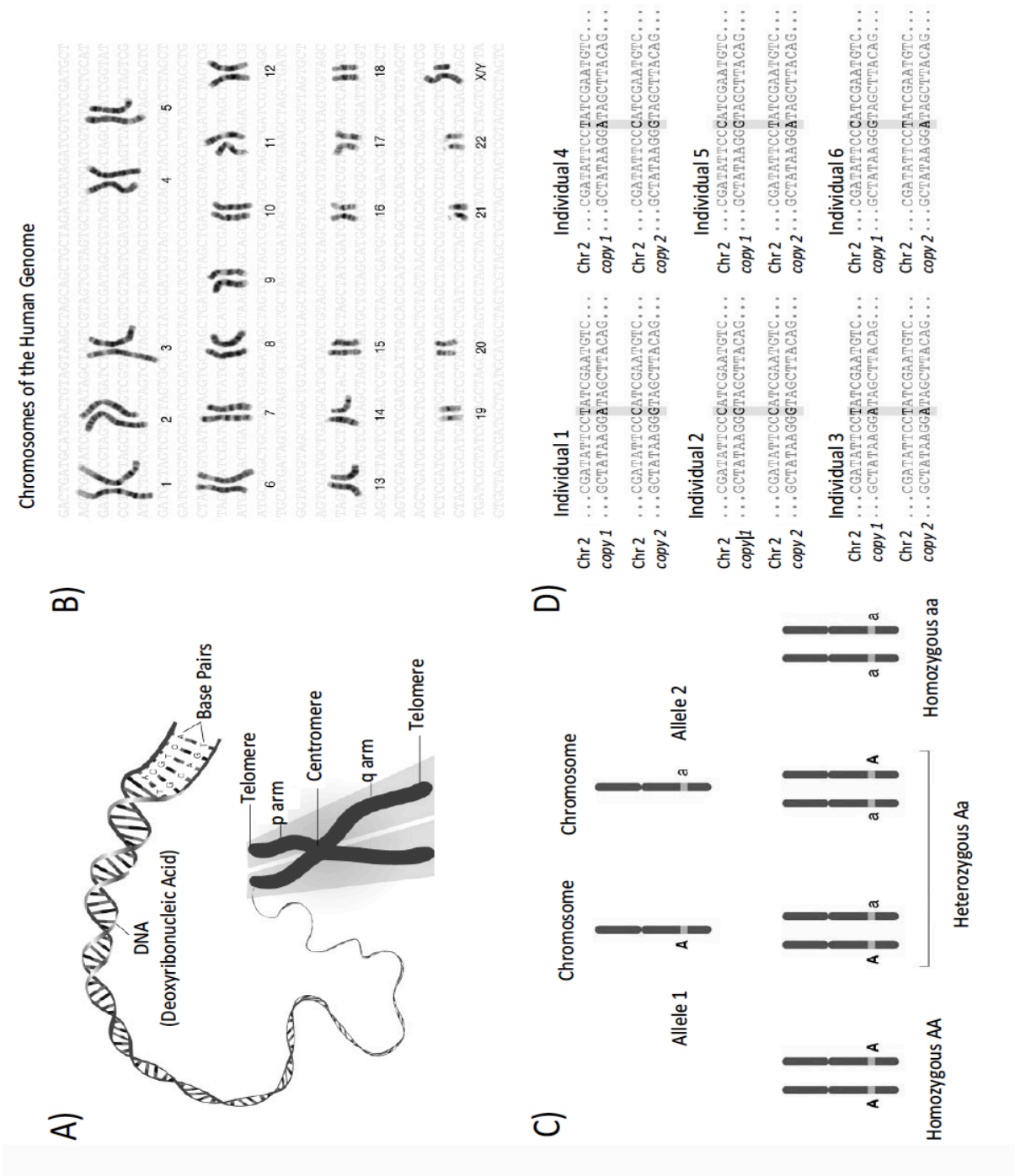

Figure 1-4: Key concepts in genetics.

(A) DNA and chromosome; B) Chromosomes of the human genome; C) Alleles and their possible configurations; D) SNP. Figure modified from www.genome.gov/Glossary. Source: (Kaakinen, 2012))

1.5.3.2 Study approaches to identify genetic risk factors

Genetic epidemiology can be regarded as a discipline closely related to traditional epidemiology, focusing on the role of genetic factors in determining health and disease, and the interplay of genes with environmental factors (Burton et al., 2005). Different analysis methods have been employed in the quest of finding genetic risk for traits, some are highlighted below.

Single marker analysis: In single marker analysis, the single variant, such as a SNP, is tested for an association with the phenotypic trait outcome of interest. These approaches further are subdivided based on whether they are hypothesis driven or are hypothesis free (Yang et al., 2007). In a hypothesis-driven approach, variants in a gene are selected for genetic association analyses based on previous evidence on biological function or potential contribution to the development of a particular trait. An example of a hypothesis-driven approach is the candidate gene. On the other hand, in the hypothesis free approach there is no prior knowledge on variants. GWAS are examples of hypothesis free approach, where genome wide variants, usually SNPs, are tested for statistical association with the trait of interest (McCarthy et al., 2008).

Multiple analysis: Unlike in the single marker analyses, which aim at associating each single genetic variant on a phenotypic trait of interest at a time, multiple analyses examine the combined effects of multiple sequence variants, a haplotype, on a phenotype. Approaches based on haplotype analysis are said to be more powerful than the single marker based ones, since the linkage disequilibrium (LD) information from multiple markers is incorporated into the analysis (Liu et al., 2008). New methods to explore gene-gene and gene-environment interactions are being developed (Mackay, 2014; Manuck et al., 2014).

1.5.3.3 Genetic studies

A trait can be due to the mutation/effect of one genetic variant (monogenic) or multifactorial (polygenic). The multifactorial or polygenic condition is the additive effect of different genes towards the trait with different levels of susceptibility; hence several genes and environment contribute to a particular phenotype. The completion of the Human Genome Project (Collins et al., 1998) and also the International HapMap Project (Gibbs et al., 2003) unearthed several millions of DNA sequence variants in the human genome. GWAS has been successful to establish genetic variants associated with different complex diseases. Since GWAS work under the common variant common disease assumption, it focuses on the identification of common variants, but it is possible that analyses of

low-frequency (0.5% < MAF < 5%) and rare (MAF < 0.5%) variants could add more information on disease risk or trait variability (McCarthy et al., 2008). Rare variants have been reported to play an important role in human diseases (Eichler et al., 2010; Gibson, 2012; Lee et al., 2014; Zuk et al., 2012). All in all, GWAS loci have a small effect on disease risk and explain very little in genetic variation of the quantitative trait; hence we cannot use its results into translating such knowledge into functional research or into clinical practice.

The advance in technology and the availability of the SNPs have made it possible to study the genetic aetiology of many complex diseases. Populations of different ancestries display different genetic architecture as such there is a need to compare the genetic loci across these population. A cross-ancestry comparison could help understand the physiology or pathways that underlies certain disease conditions, such as obesity, around the world. Secondly the cross-ancestry comparison would help to pinpoint specific obesity-susceptibility genes through fine mapping. GWAS often discovers a lot of loci, with some harboring multiple genes sometimes with no genes at all; this hinders the ability to do follow-up studies for functional research.

By taking advantage of differences in genetic architecture across different ancestries, these genetic loci can be narrowed down hence unearth the causal genetic variants and/or causal genes behind the GWAS association (Lu et al., 2013). In Africa deliberate efforts have been made to help understand genetic variation within African populations and across other ancestries, and also to understand the genetic and environmental factors to complex disease susceptibility through initiatives such as the Southern African Human Genome Project (Pepper, 2011), the African Genome Variation Project (Gurdasani et al., 2015) and the National Institutes of Health (NIH) and Wellcome Trust funded Human Heredity and Health in Africa (H3Africa) Consortium (H3Africa, 2014).

Family history has long been used in risk assessment of some NCDs such as CVD. It is an indicator of CVD risk in future generations (Myers et al., 1990). This link between the cardiovascular profile of one's ancestors and someone's own risk of cardiovascular disease implies that genetic variation within or between populations as a contributor to the cardiovascular disease risk. Results from twin and family studies designs have revealed existence of genetic influence to human variability in NCDs risk factors.

Advances in genome wide association studies (GWAS) have been successful in unearthing many genetic loci associated with NCDs and NCD risk factors such as lipids, blood pressure, diabetes and

obesity (Ehret et al., 2016; Locke et al., 2015; Morris et al., 2012; Willer et al., 2013). For instance, the most recent meta-analysis of adult BMI reported 97 loci, near or in genes like *APOE* and *FTO,* being robustly associated with BMI (Locke et al., 2015). *APOE* is strongly linked with plasma LDL-C concentration and LDL-C is one of the modifiable risk factors for CVD (Assmann et al., 1998). The latest and largest meta-analysis of coronary heart disease (CHD) GWAS (Deloukas et al., 2013), including 63,746 CHD cases and 130,681 controls, identified 15 novel variants for CHD. Adding to previously known variants puts the number of independent genome-wide significant SNPs for CHD over 50 (Deloukas et al., 2013).

Many loci have been reported to be associated with blood pressure in predominantly Caucasian population studies. A total of 66 loci have been reported to be robustly associated with blood pressure so far. Of the 66 loci, 13 were associated with SBP only, 12 for DPB only and 41 for both traits (Ehret et al., 2016). Tiago and colleagues reported that a *CY11B2* gene polymorphism was associated with SBP in the newly diagnosed hypertensive patients of African ancestry in South Africa (Tiago et al., 2003).

Table 1-3 shows different ranges of heritability estimates from studies which looked at individual risk factor as reported by Rankinen and colleagues (Rankinen et al., 2015). To fully understand the genetics of NCDs, both emerging and well-known risk factors should be investigated. In this study, we investigated the genetics of BMI, with a heritability range of 25-70%, as a measure of obesity, which is one of the common risk factors for NCDs. Though BMI is reported to be highly heritable as shown in Table 1-3, only about 2.7% of phenotypic variance has been explained by known genetic risk variants as shown in Table 1-4.

Table 1-3: Commonly Reported Heritability Levels for NCD Risk Factors

Phenotype	Estimated Heritability, %[&]
Body composition	
Body mass index	25–70
Body fat	25–40
Abdominal obesity	40–55
Insulin/glucose	
Fasting glucose	10–75
Fasting insulin	20–55
Insulin resistance	46–90
Lipids	
Triglycerides	25–60
*LDL-C	25–60
[@]HDL-C	30–80
Total cholesterol	50–60
Blood pressure (BP)	
Systolic BP	20–70
Diastolic BP	10–50
Hypertension	50

*LDL-C, low-density lipoprotein cholesterol

[@] HDL-C, high-density lipoprotein cholesterol

[&] The heritability ranges are based on different studies as reported in a systematic review by Rankinen and colleagues (Rankinen et al., 2015)

Table1-4: Summary of GWAS Loci for NCD Risk Factors (Modified from Rankinen et al., 2015)

Trait	No. of participants	No. of Loci*	% Variance Explained by Loci	Reference
BMI	339224	97	2.7	(Locke et al., 2015)
CAD	67376 cases 142664 controls	45	10.6	(Deloukas et al., 2013)
CRP	81870	18	≈ 5	(Dehghan et al., 2011)
BP	342415	66		(Ehret et al., 2016)
Diastolic only		12	3.36	
Systolic only		13	3.46	
Both SBP and DBP		41		
Hypertension	203056	11	0.90	(Ehret et al., 2011)
Lipids‡	188577	157		(Willer et al., 2013)
HDL-C		62	≈12-14	
LDL-C		30	≈12-14	
TC		40	≈12-14	
Triglycerides		32	≈10-13	
T2DM	34840 cases 114981 controls	65	5.7	(Morris et al., 2012)

CAD, coronary artery disease; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T2DM, type 2 diabetes mellitus

* Number of loci as specified by authors of each meta-analysis. Thus, loci may or may not have been significant at the genome-wide level of $p \leq 5*10^{-8}$

‡ For individual lipids and lipoprotein traits, the number of loci represents those whose strongest association was for the specified trait. As a multiple SNPs within the same loci may have reached the genome-wide significance, the number of loci across individuals traits amounts to > 157.

The prevalence of obesity varies across populations and there might be population-specific genetic variation playing a role (Locke et al., 2015; Lombard et al., 2012; Minster et al., 2016; Pillay et al., 2015; Wen et al., 2012; Zhu et al., 2014). In an effort to understand the genetic overweight and obesity risk many genetic studies have been done predominantly in populations of Caucasian ancestry. The first locus to be associated to obesity was reported in 2007 (Frayling et al., 2007). This locus is found within the intronic region of the *FTO* gene in chromosome 16.
By 2016, GWAS have been successful in identifying common genetic susceptibility variants for obesity in more than 97 loci, but the association varies among ethnicities (Locke et al., 2015; Silva et al., 2013; Speliotes et al., 2010). Different body composition measures, which are also risk factors for obesity, have also been reported to be associated with different genetic risk variants (Lu et al., 2013), as shown in Figure 1-5.

Most obesity associated SNPs have been replicated in people from European, Asian and Hispanic populations but inconsistent results have been reported in African populations with both positive and no associations being reported (Fesinmeyer et al., 2013; Fulford et al., 2015; Hester et al., 2012; Shinozaki et al., 2012; Silva et al., 2013; Zhou et al., 2013). In a recent GWAS BMI systematic review in Africa, Yako and colleagues reported that more than 300 SNPs have been studied for association with BMI in 42 genes. Most of these studies were not replicable and though about 36 polymorphisms have been previously validated through GWAS in non-African populations such as Caucasian and East Asian populations, only *FTO* and *MC4R* polymorphisms have been be significantly associated with obesity in black South Africans, Ghanaians and Nigerians (Yako et al., 2015).

Another recent study in Samoans has reported a common variant rs373863828 in the *CREBRF* gene to have a positive selection effect on the Samoan population. This variant is very rare in other populations but common in Samoans (Minster et al., 2016). There is still a long way to go to fully understand heritability and progression of obesity in African populations. Since African-ancestry populations display a wide range of genetic diversity and different LD structure, understanding genetic variation in the genes associated with overweight and/or obesity in African population promises to provide new understanding into their association with obesity (Adeyemo et al., 2010). Much as most research has been focused to understand genetics of obesity in adults, understanding the genetics risk factors of obesity in childhood paves a way to develop preventive measures in early

life. So far a total of 15 adult BMI associated genetic loci have also been implicated in childhood obesity (Locke et al., 2015; Mei et al., 2012; Stergiakouli et al., 2014; Warrington et al., 2015).

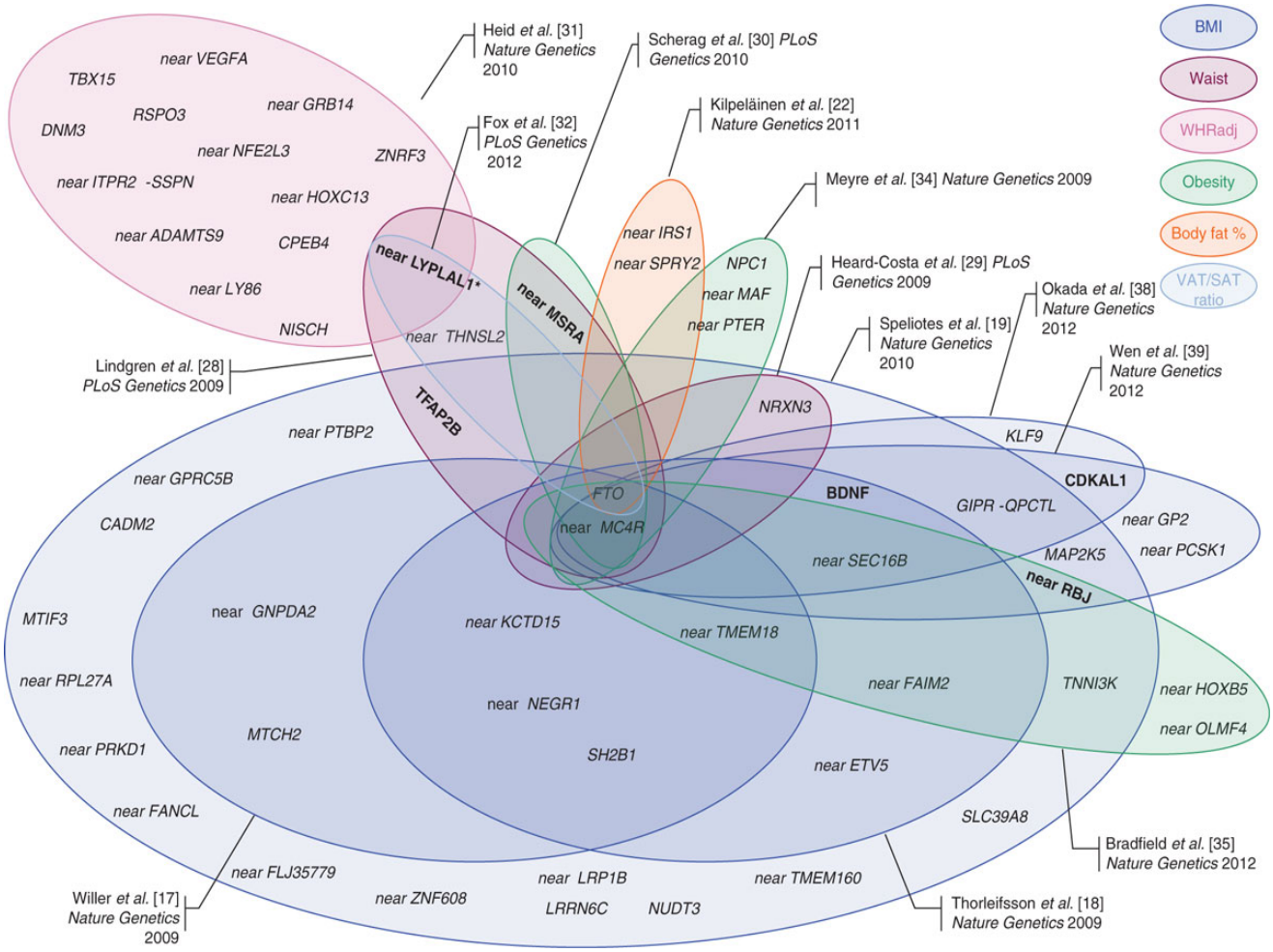

Figure 1-5: Common obesity susceptibility loci. Source: (Lu et al., 2013))

Obesity-susceptibility loci discovered in four waves of GWAS for BMI (blue), in one genome-wide meta-analysis for body fat percentage (orange), in two waves of GWAS for waist circumference and waist-to-hip ratio (pink), and in two GWAS for extreme and early onset obesity (green). Each Venn diagram represents the loci of one paper, except for papers that discovered only one locus (i.e. FTO and near-MC4R) for which no Venn diagram has been drawn. Figure retrieved from http://genomemedicine.biomedcentral.com/articles/10.1186/gm459

Though GWAS has been successful to establish SNPs associated with BMI, there are still issues some of which are: missing heritability, very small effects size and the attributed risk to the population is very small. Hence there is a need to focus on gene and environment interaction and to find rare variants with strong effects, which are not found through GWAS. The investigation of copy-number variations (CNVs) and whole genome sequencing (WGS) technologies would help to uncover those genetic variants not uncovered by GWAS (Gluckman et al., 2010).

1.6 DOHaD and Obesity

1.6.1 The life-course approach to obesity

According to the theory of the Developmental Origin of Health and Diseases (DOHaD), exposure to some conditions pre-pregnancy and during the neonatal and childhood periods provide an essential opportunity to mitigate individual's sensitivity to, or risk of developing diseases, such as diabetes, later in life (Barker, 2012; Macaulay et al., 2014; Silveira et al., 2007). The DOHaD paradigm emphasizes on a life-course approach to the understanding of disease development and progression. A life-course approach of understanding CVD and other NCDs development and progression involves studying the long-term effects of physical and social effects in combination with genetic exposures during gestation, childhood, adolescent, young adulthood and later. This approach involves exploring the biological, physiological and behavioral pathways that operate within an individual's lifetime as well as across generations and how they influence the development of chronic diseases (Barker, 2004; Barker, 2012; Gluckman et al., 2010). Its main goal is to understand how early life factors influence the risk of chronic diseases later in life. In this approach a given risk factor exposure in early childhood and the accumulation of risk along the life-course can increase susceptibility to risk of obesity and other later health outcomes such as hypertension (Aboderin, 2002; UNSSCN, 2006a, 2006b). Kral compared the rate of obesity of children born when their mothers were morbidly obese (with average BMI of 48 kgm^{-2}) to children born from the same mothers after the women had lost weight (with average BMI of 31 kgm^{-2}). The rate of obesity was much lower in children born later compared to their older siblings (Kral et al., 2006).

Figure 1-6 shows that the risk of obesity accumulates with age and is influenced by factors acting at all periods of the life-course. The main factors associated with different growth periods include the following:

- *Foetal life:* Foetal growth, maternal nutritional status, maternal weight status, maternal smoking, socio-economic position at birth, birth weight (birth length/head circumference), see Figure 1-7.

- *Infancy and Childhood:* Rapid growth, breastfeeding and complementary feeding practices, sleeping patterns, maternal diabetes, infectious diseases, unhealthy diet, lack of physical activity, obesity and socio-economic position.
- *Adolescence:* Unhealthy dietary practices, physical inactivity and sedentary behaviors including TV viewing, tobacco smoking and harmful alcohol consumption.
- *Adult life:* Poor diet, physical inactivity, tobacco smoking, harmful alcohol consumption, biological risks, socio-economic status, and environmental conditions. The proportion of overweight/obese increases significantly and a stage when most NCDs manifest.

1.6.2 Early life risk factors for obesity

The early life influences beginning with the conditions a foetus is exposed to in the uterus up to the first few years of life shape the trajectory of weight gain and body fatness throughout the life course as described in the Figure 1-6 (Monasta et al., 2010) . Both prenatal (mother's smoking, weight gain, blood sugar levels) and postnatal (infant's weight gain and irregular sleeping patterns) factors increase the obesity risk in both childhood and adulthood. In addition, the genetic makeup of both the infant and mother may influence risk.

Despite some interventions, adult obesity has proved hard to treat as and such there is a need to come up with interventions in early life to mitigate future health risks that come with childhood obesity (Livhits et al., 2012; Loveman et al., 2011). A systematic review and meta-analysis on the association between BMI, sex and cardiometabolic risk factors reported that overweight or obese children have higher blood pressure, higher levels of triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol, and fasting insulin and insulin resistance (Friedemann et al., 2012).

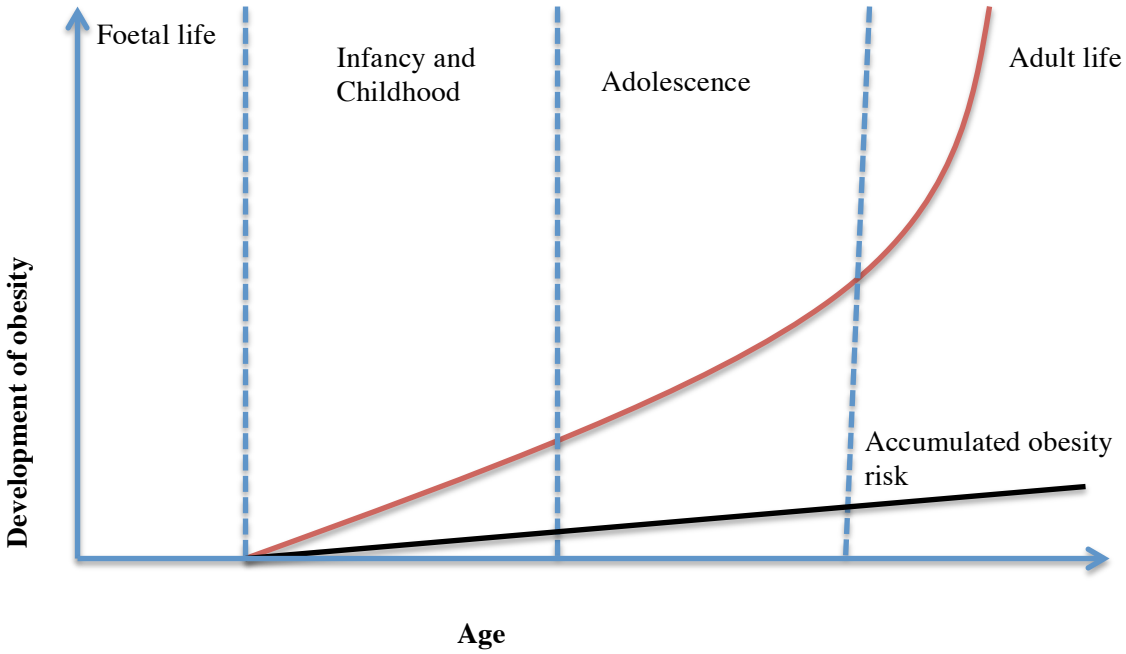

Figure 1-6: A Life-Course approach to obesity risk.
(The bottom line on the graph represents genetic susceptibility to obesity and the top line represents the environmental exposures to obesity risk). Source: modified from (Aboderin, 2002; Darnton-Hill et al., 2004)

DOHaD explicates how an unfavorable intrauterine environment due to maternal obesity and other factors increases the risk of obesity in the offspring. In Figure 1.7, the pathway highlighted in red shows how maternal obesity can lead to obesity in the child. Overweight or obese women are likely to develop gestational diabetes. Diabetes leads to higher birth weight (big babies) this leads to rapid growth catch-up which is associated with obesity both in children and adults (Monasta et al., 2010; Prescott, 2015).

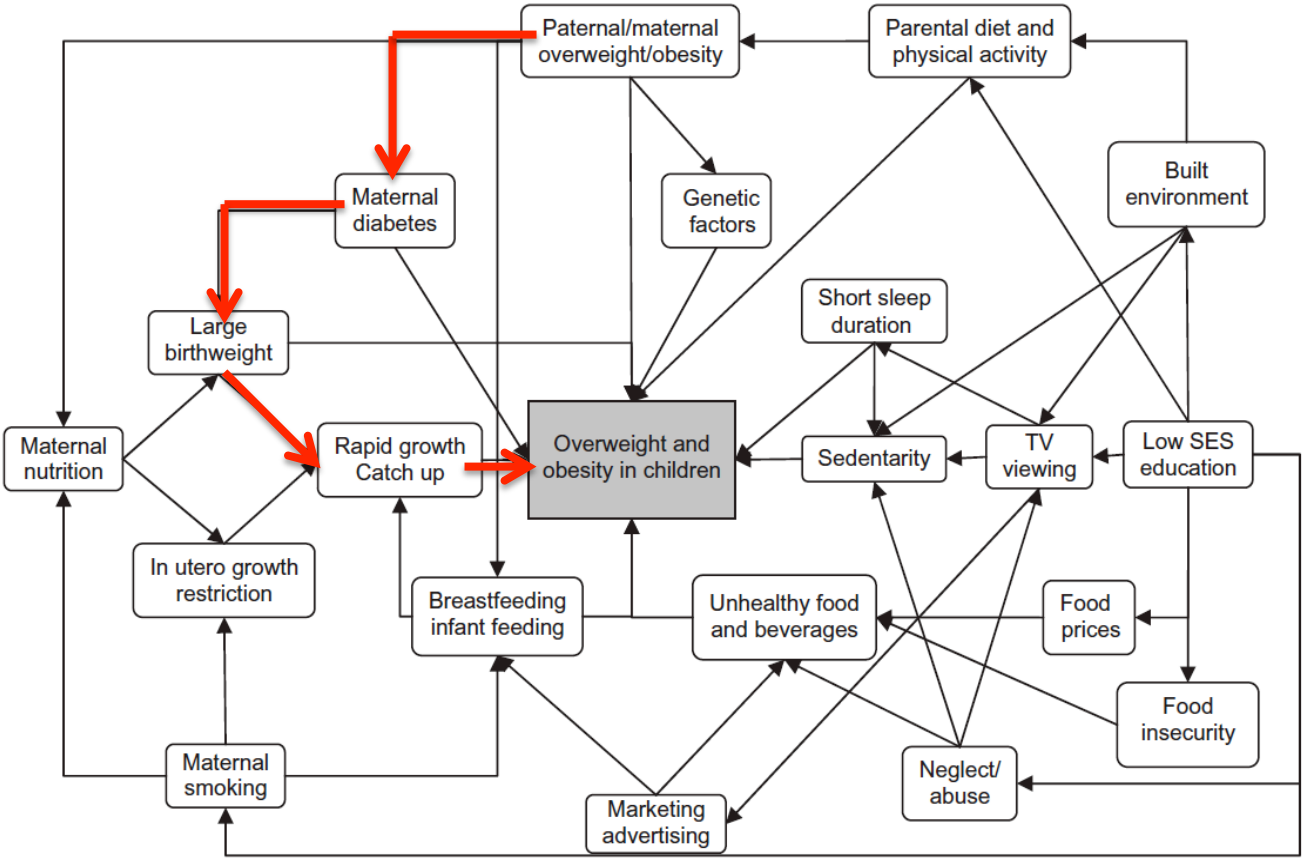

Figure 1-7: A path diagram for the potential early life factors predictors of overweight and obesity in children

Source: modified from (Monasta et al., 2010)

1.7 Longitudinal Studies

1.7.1 Longitudinal data analysis methods

In order to study obesity across the life-course discussed in Section 1.6 above, longitudinal approaches are employed. A longitudinal study can be defined as a study where data of interest is collected from the same subject at multiple follow up time points (Diggle et al., 2002). It generally yields multiple or repeated measurements on each subject as opposed to cross-sectional where each individual has data collected at a single point only. A cohort study involves observing the same study participants over the study period. This helps researchers to study how a particular variable of interest changes over time. Variations in overweight or obese onset across the life-course between

individuals and overtime due to biological changes, social and behavioral influences have been reported in some populations (Howe et al., 2015). Using a life-course approach would help us to understand when we observe the age or period with peak obesity incidences hence this age/period may serve as a critical period for preventative intervention strategies.

Longitudinal data analysis methods are widely used in cohort studies to capture effects that vary overtime unlike cross-sectional data, which fails to separate age effects from the birth-cohort effects. Birth cohort effects are factors that differentiate one group of studies from the other due to the time or year of birth. Appropriate methodology must be selected when performing longitudinal data analysis. There is a need to choose the method that will account for the correlation between measurements from the same subject. Ignoring this may lead to incorrect inferences, bias results or less precise estimates. You also need to choose an appropriate method to deal with missing data. If it is left unattended missing data may lead to incorrect conclusions.

Mixed models, which are statistical models containing both fixed effects and random effects, are mostly used to analyze longitudinal data since they can handle missing data. Fixed effects describe the influence of known measured covariates such as time or sex, where these effects are assumed to hold in the whole population of individuals. Random effects measure the influence of the known variables where effects are assumed to be different across individuals. In genetic association studies, genetic variations that are time-dependent may help to predict those that will go on to develop the disease before they starting showing symptoms (Smith et al., 2010). Mixed models assume that the incomplete data is missing at random, that is, the reason for missingness can be related to the previous observed data but not related to the missing data itself. Tiwari et al. 2011 identifies the main goals for longitudinal studies as to characterize the pattern subject over time and to find out the impact of the important covariates in the model on these patterns (Tiwari et al., 2011). These covariates may be time dependent or not. The LCGMM (Section 2.4), have been used to characterize variation in BMI growth patterns and their associated future health risks in different populations before (Albrecht et al., 2013; Attard et al., 2013; Börnhorst et al., 2016; Boyer et al., 2015; Haga et al., 2012; Howe et al., 2015; Pryor et al., 2011; Wang et al., 2016; Ziyab et al., 2014).

1.7.2 Longitudinal genetics of obesity

Most genetic association studies investigating the correlation between genetic variants and disease risk make use of cross-sectional phenotype data (Chiu et al., 2016; Kerner, North, et al., 2009; Locke et al., 2015; Smith et al., 2010; Ware et al., 2015; Wu et al., 2014; Yako et al., 2015). The association between genetic variants and phenotype such as BMI may vary according to time; the big question remains whether BMI is influenced by the same gene(s) over time or whether age-specific genetic effects exist. The longitudinal approach was used in the replication study done by Mei et al. 2012. The study looked at the longitudinal replication studies of GWAS risk SNPs influencing the BMI over the course of childhood and adulthood (Mei et al., 2012). Mei's results showed that variants near or at certain genes were associated with BMI at different age ranges. For example, variants near or at *BDNF FAIM2*, *TFAP2B* and *FTO* associated with childhood BMI while *MAP2K5, MTCHM2* with adulthood and *KCTD15* with both. The study found that variants of genes *FAIM2* and *MAP2K5* affect BMI depending on age and that the *FTO* gene influences both adulthood and childhood BMI (Choh et al., 2014; Mei et al., 2012; Zhao et al., 2009). Another 12 year longitudinal study in Chinese adults showed that *FTO* rs8050136 and *GNPDA2* rs10938397 SNPs, rather than *MC4R* rs17782313, predicted central obesity over time (Cheung et al., 2011).

Very few studies have made use of longitudinal data in genetic association studies due to its computational demand and lack of availability of data. A large number of parameters must be estimated at each iteration in the regression models, which demands significant computational resources (Chiu et al., 2016; Kerner, North, et al., 2009; Wu et al., 2014). This makes it difficult to use traditional genomic analytical approaches and finding other methods to analyze such data has become necessary. One way is to redefine the longitudinal phenotype data to reducible variables, where the longitudinal data with many time points can be presented into two or three subgroups (Kerner, North, et al., 2009; Londono et al., 2013; Musolf et al., 2014; Sakai et al., 2010; Tan et al., 2014).

Different analytical approaches have been used before in genetic association with longitudinal phenotype for instance Chiu and colleagues 2012, looked at four analytical methods; generalized estimating equations (GEEs), LCGMM, linear mixed-effect (LME), and variance components (VC) to find which method increased genetic variant signal identification associated with blood pressure/hypertension (Chiu et al., 2016). The latent class model applying the LME approach was reported to be a better approach than others. It was concluded that if properly used these approaches

would increase statistical power, decrease computational coast, decrease phenotype heterogeneity and help to find genetic variants influencing clinically important trajectories within the population (Chiu et al., 2016).

This study takes advantage of group-based trajectory modeling to redefine longitudinal BMI phenotypes for longitudinal genetic association. The method takes heterogeneous longitudinal data and analyses it into homogeneous subgroups, which would help to determine, or ability to detect genetic differences in BMI trajectory groups. Since these trajectories represent different time of either increase or decrease in BMI or disease onset, the association between trajectories and genetic variants would help to pin point the growth period these associations are strongest (Warrington et al., 2013). Longitudinal phenotypes may help us to understand how phenotypes and genotype variants associate, hence greater understanding of variants and covariates of time. Using growth mixture models approaches, each individual's phenotype data is described by a particular growth trajectory curve using the estimated Bayesian posterior probabilities (Londono et al., 2013). We can identify a high-risk trajectory and stratify our genetic analysis according to the trajectory membership. This could be useful to detect rare mutations in common complex diseases. It could also help to improve the precision in estimating population impact of genomic risk (Kerner, North, et al., 2009). Kerner and colleagues argue that cross-sectional and case-control study designs do not allow exploration of risk factors due to gene-environment interaction that might influence development of a trait over time (Kerner, North, et al., 2009).

The other approach utilized in this study is the use of a genetic risk score, which is constructed by summing up the number of risk alleles for disease prediction. The genetic risk scores are not affected by background noise such as age, ethnicity and environment, and not affected by the multiple testing problems unlike when using the individual genetic markers (Cooke Bailey et al., 2016; Monnereau et al., 2016; Smith et al., 2015). The risk score is used as an exposure in regressions and other longitudinal data analysis tools.

1.8 Conclusion

This chapter has highlighted the importance of looking at NCDs in LMICs where there has been an increase in prevalence of NCDs and mortality rate due to NCDs. It has also reported the paucity of longitudinal studies on obesity and its genetic influence from childhood to early adulthood in LIMCs. Risk factors for CVDs and objectives for the study have been put forward. The next chapter looks at major methodological considerations in this study.

Chapter 2: Overview of Methodological Approaches

This chapter presents the study setting and design, analysis plan, ethical considerations and overview of the methodological approaches used in the study. This study utilized data from the Birth to Twenty Plus cohort, which is Africa's largest and longest running birth cohort. Children born in 1990 in a South African urban township, namely Soweto in Johannesburg and have since been followed up to date.

2.1 Study Setting and Design

The current study was conducted in metropolitan area of Soweto in Johannesburg, South Africa, just 15 kms from the Johanneburg Central Business District (CBD). Soweto stands for South West Townships, which was developed mostly to accommodate black people who were displaced from their homes in Johannesburg after passing the Group Areas Act of 1950, the racial segregation policy on residential areas, by the South African government (Lewis, 1966; Mesthrie, 1993).

In 1990, Soweto was made up of 34 suburbs and covered approximately 200 km^2, with a population estimated at 3.5 million (Richter et al., 2004). Soweto had a high population growth speared by rapid, unplanned urbanization in an ethnically and socioeconomically diverse community with limited health and other essential social amenities. The initial aim of the Birth to Twenty Plus cohort, formerly known as Birth to Ten cohort (Richter et al., 1995; Yach et al., 1990) and later Birth to Twenty cohort (Richter et al., 2007)**,** was to explore socioeconomic, environmental, biological, psychosocial and physical measures associated with health and development of urban children. But after age of ten years the cohort started to explore other factors such as biochemistry, bone health, and genetic factors related to cardio-metabolic disease risk (Richter et al., 1999; Richter et al., 2004; Richter et al., 2007). Only mothers within the study area who were expected to deliver between April and May 1990, and who were to remain within the study area six weeks after delivery were invited to participate. Only 3273 (1682 girls and 1591 boys) children met the inclusion creteria and were recruited in the cohort (Richter et al., 2007).

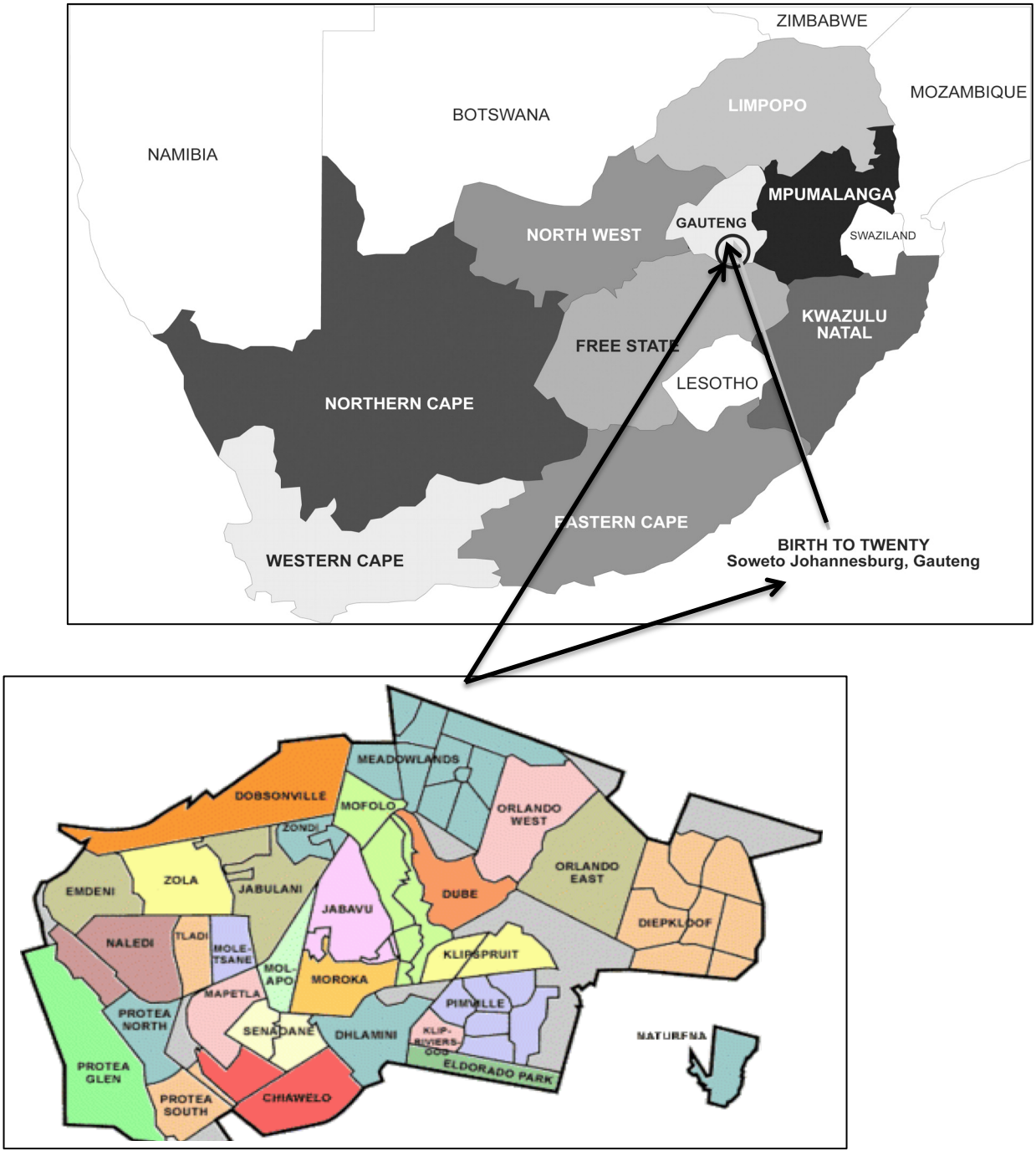

Figure 2-1: Map of Soweto-Johannesburg in Gauteng Province, South Africa

Modified: (Richter et al., 2007).

Trained interviewers collected baseline data through questionnaires from pregnant women attending antenatal clinics in the study between March and May 1990. Data collected following delivery were maternal demographic details (such as contact details, parity and age) and the details of the infant (such as date of birth, infant sex, ethnicity and birth weight). The participants have been followed up to date using different means of communication. An attrition rate of less than 3 % has been reported in this cohort, detailed cohort profile with recruitment and cohort attrition details has been described elsewhere (Richter et al., 2007). The phenotype data used in this study have been described in Emprical Chapters; Sections 3.4, 4.4, and 5.4. The same standardized queistionnaire was used to collect data in the cohort. All the three manusripts used the same data.

Our study made use of the DNA samples and anthropometric/phenotype data collected from the participants, which includes weight, height and blood pressure. We utilised Metabochip data as part of the AWI-Gen Collaborative Centre (AWI-Gen) study under the Human Heredity and Health in Africa (H3Africa) (H3Africa, 2014; Ramsay et al., 2016). AWI-Gen's main focus is to build capacity for research and to find genetic, epigenetic and environmental factors contribute to the risk factors for obesity and associated cardio-metabolic disease (CMD) in sub-Saharan Africa. The H3Africa Initiative aims at exploring environmentl and genetic factors associated with NCDs in African populations.

The Bt20+ cohort research protocol was submitted for ethics clearance for data and DNA collection to the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (Wits HREC) and all routine procedures have been approved and the work done related to this PhD study was also approved, (Reference number: M1411115), Appendix 2. Participants or their parents/caregivers when the participants were minor gave informed consent at the beginning of each data collection session throughout the study.

2.2 Genotype Data

2.2.1 DNA preparation

The genotype data was generated by other students as part of the larger project that this study forms part of (Sahibdeen, 2016). DNA at age 13 years was extracted using a salting-out technique (Miller et al., 1988) quantified using absorbance spectroscopy, and normalized to a concentration of 50ng/ul for genotyping.

2.2.2 Genotyping

Sample

1248 black children/participant samples from the Bt20+ cohort were randomly sampled for genotype data. Genotyping was done at UC Davis Genome Center (USA). Genotyping was done using Illumina CardioMetabochip (Voight et al., 2012)

CardioMetabochip

Metabochip is a customized genotyping chip for replication and fine mapping of metabolic, cardiovascular and anthropometric trait loci, previously identified through GWAS and meta-analyses. It also contains other markers such as those from X and Y Chromosomes for sex verification (Voight et al., 2012)It also includes low frequency variation content identified from the 1000 Genomes project (Siva, 2008). It contains 196,725 SNP markers related to 23 traits concentrated in 257 genomic regions.

Table 2-1: Traits on the Illumina CardioMetabochip. Source Modified from (Voight et al., 2012)

Traits represented on the Metabochip			
1	Type 2 diabetes	13	T2D early onset
2	Coronary Artery Disease	14	Mean platelet volume
3	HDL cholesterol	15	Platelet count
4	LDL cholesterol	16	White blood cells
5	Triglycerides	17	Total cholesterol
6	Body Mass Index	18	Body fat percentage
7	Waist to hip ratio	19	Height
8	Fasting glucose	20	Waist circumference
9	Diastolic blood pressure	21	2 hour glucose
10	Systolic blood pressure	22	Glycated hemoglobin
11	QT interval	23	Fasting insulin
12	T2D age of diagnosis		

2.2.3 Quality control

Quality Control (QC) was performed following the genetic data QC protocol as suggested by Anderson (2010) (Anderson et al., 2010) using PLINK 1.9 as explained in Section 5.4.2.

2.3 Data Analysis: Methodological considerations

The main analytical techniques employed in this analysis of longitudinal data include group-based modelling, multilevel mixed models, risk scores and mediation analysis to understand the variation of BMI developmental patterns and the genetic risk factors of obesity.

BMI trajectories are used as a redefined phenotype of BMI, and genetic risk score represents a cumulative effect of genetics. BMI measures at multiple time points from birth to 18 years and Metabochip SNP data in the Bt20+ cohort were used. BMI developmental patterns emerging from longitudinal data were assessed, see sections 2.4.1, 2.4.2 and 3.5.1 .

Next, individuals were categorized into different trajectories. Finally SNPs or genetic risk variants that were associated with these trajectories were identified, to assess the role genetics play in predicting trajectories membership (Londono et al., 2013). Every individual was assigned a class based on their Bayesian posterior probability to the pattern they had the highest likelihood of belonging to (Muthen, 2001; Nagin, 2005). The trajectory memberships were graded by increasing risk of childhood obesity and used as both exposure and outcome variable in subsequent analyses.

Group based models can be implemented using SAS PROC TRAJ program, with a STATA plugin TRAJ (Jones et al., 2001; Nagin, 2005; Nagin et al., 2010) and the Mplus software (Muthen, 2001; Muthén et al., 2000; Muthén, 1998-2012). Mplus and LCGMM were used since they allow variation in the intercept and slope in both within the class (inter-individual differences) and across classes while SAS *Proc Traj* allows only across classes variation.

2.3.1 Trajectory analyses

In a homogeneous population where the whole population is described by the same growth curve a parametric equation of $f(\boldsymbol{y}, \lambda)$ may be assumed for BMI, where $\boldsymbol{y} = (y_1, y_2, y_3 \ldots y_T)$ is longitudinal sequence of observed BMI measures across time T periods i.e. $\boldsymbol{y} = (bmi_5, bmi_7, bmi_8 \ldots bmi_{18})$. In an event where more than one BMI trajectory exist in a population, we employ growth mixture modelling techniques, which classify individuals with similar growth curves together. Using growth mixtures models, intra-individual change overtime can be described as a function of group

membership (C_i) of belonging to K^{th} class is determined based on similarity in initial values of BMI at 5 years (intercept) and change in BMI overtime (slope). Now the model can be presented as:

$$f(\boldsymbol{y}) = \sum_{k=1}^{K} \Pr(C = k) \Pr(\boldsymbol{Y} = \boldsymbol{y} | C = k) = \sum_{k=1}^{K} p_k f(\boldsymbol{y}, \lambda_k) \qquad (1)$$

The main goal in the model in equation (1) is to estimate p_k, which is the probability of an individual belonging to class k, BMI development pattern, as a function of parameter λ_k, where λ_k dependent on time.

Using this approach, individuals in a clinically important BMI trajectory, for instance those in an obese trajectory, can be identified. Then different demographic and other profiles can be compared to those individuals in a referent group, the normal BMI trajectory. The trajectory membership can also be used to predict some distal outcome later in life. The early life factors and genetic factors associated with the clinically important pattern can be determined.

2.3.2 Thesis overall analysis plan

This study has tackled three areas; firstly, it looked at heterogeneity of life-course BMI development and early life factors driving these variations. Then the study investigated the life-course BMI and the risk of elevated blood pressure and obesity later in life and lastly, looking at the additive genetic effect for BMI and determining whether genetic risk of obesity in early adulthood was mediated by early weight gain or rapid growth.

Mplus (Mplus, USA) (Muthén, 1998-2012) was used to find distinct BMI trajectories. Trajectories may capture certain characteristics of the subgroups within the population with unique genetic and environment risk factors. They may also capture variability or stability of the phenotype over time (Kerner, North, et al., 2009). After identification of these childhood adiposity trajectories different analyses followed as shown in Figure 2-1. The next three chapters present empirical results from this study, where the analysis plan was implemented.

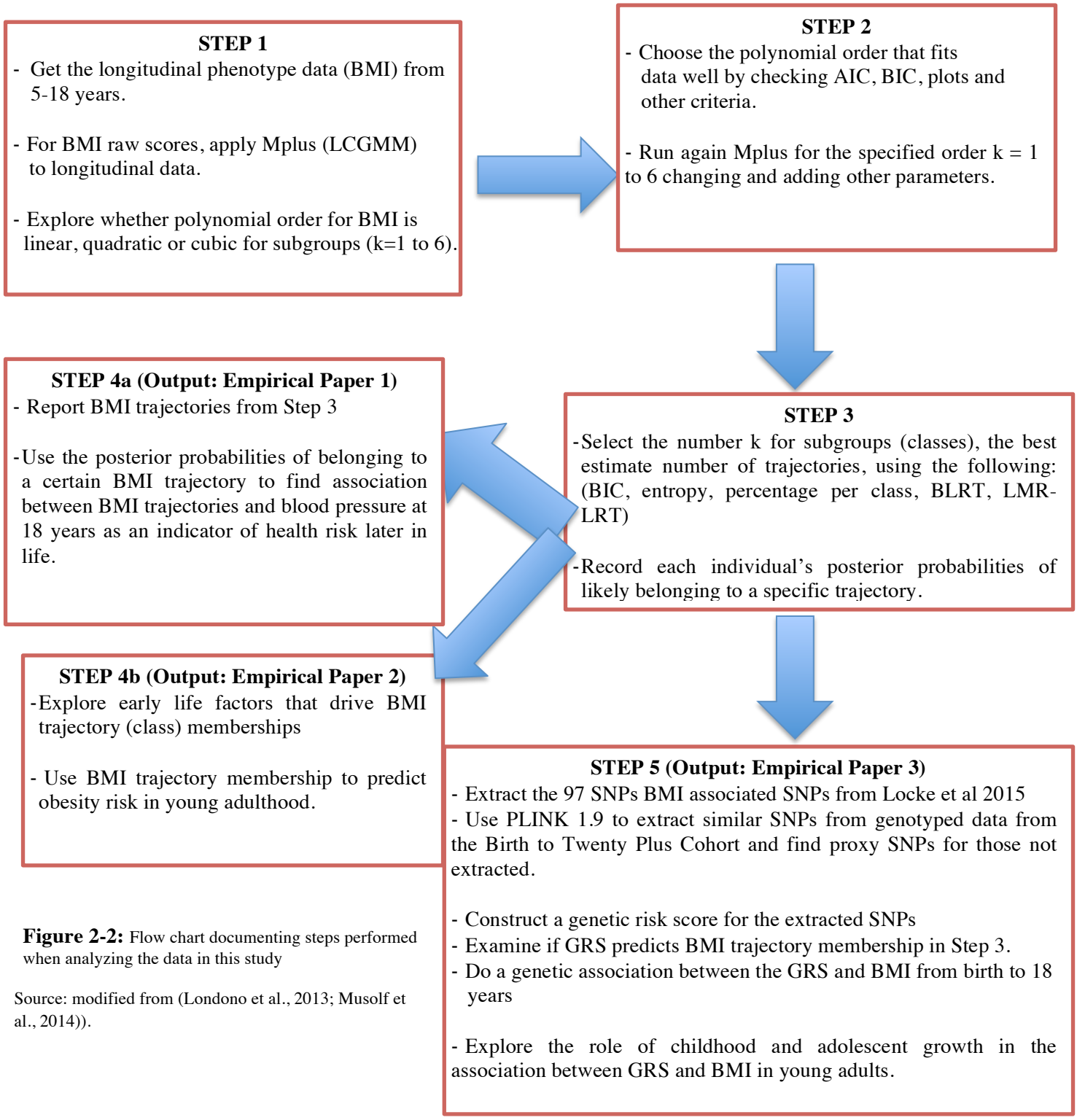

Figure 2-2: Flow chart documenting steps performed when analyzing the data in this study

Source: modified from (Londono et al., 2013; Musolf et al., 2014)).

Part 2

Empirical Chapters

Chapter 3

Childhood Adiposity Trajectories are Associated with Late Adolescent Blood Pressure : Birth to Twenty Plus Cohort

Published in *BMC Public Health*. 2016;16:665.

Chapter 3: Childhood Adiposity Trajectories are Associated with Late Adolescent Blood Pressure: Birth to Twenty Plus Cohort

3.1 Preface – Empirical Paper 1

This chapter was published as an empirical research paper as follows: Munthali RJ, Kagura J, Lombard Z, Norris SA. Childhood adiposity trajectories are associated with late adolescent blood pressure: birth to twenty plus cohort. *BMC Public Health*. 2016;16:665.

The manuscript's main contribution to the overall aim of the thesis is to identifying distinct classes of BMI trajectories in boys and girls. It was important to answer this question since identifying these trajectories would help to identify individuals who belong to BMI trajectories characterized by overweight and obesity. These trajectories were then implemented to identify those who are at high risk of developing deleterious health outcomes such as elevated blood pressure in future. We utilized data from 5 to 18 years, in black South African children from the Bt20+. We analyzed boys and girls separately. Only individuals with at least two BMI data points were included to characterize BMI trajectories using the LCGMM. Multinomial logistic regression was used to find relative risk of BMI trajectory membership to elevated blood pressure at 18 years old of age.

The individual contributions by the candidate and any co-authors to the work are as follow: R.J.M, J.K, Z.L and S.A.N carried out the initial conceptualization and design of the study. RM led the study, conducted the initial analyses and interpreted the data, drafted the initial manuscript, and approved the final manuscript as submitted in close collaboration with J.K, Z.L and S.A.N who gave guidance throughout the study from conception to the approval of the final draft, reviewed and edited the data analysis plan and the manuscript for intellectual content. All authors read and approved the final manuscript as submitted.

3.2 Abstract

Elevated blood pressure in childhood is a risk factor for adult hypertension which is a global health problem. Excess adiposity in childhood creates a predisposition to develop adult hypertension. Our aim was to explore distinct sex-specific adiposity trajectories from childhood to late adolescence and examined their association with blood pressure. Latent Class Growth Mixture Modeling (LCGMM) on longitudinal data was used to derive sex-specific and distinct body mass index (BMI: kg/m^2) trajectories. We studied 1824 black children (boys =877, girls = 947) from the Birth to Twenty Plus cohort (Bt20+) from Soweto, South Africa, and obtained BMI measures at ages 5 through 18 years. Participants with at least two age-point BMI measures, were included in the analysis. Analysis of variance (ANOVA), chi-square test, multivariate linear and standard logistic regressions were used to test study characteristics and different associations. We identified three (3) and four (4) distinct BMI trajectories in boys and girls, respectively. The overall prevalence of elevated blood pressure (BP) was 34.9% (39.4% in boys and 30.38% in girls). Boys and girls in the early onset obesity or overweight BMI trajectories were more likely to have higher BP values in late adolescence. Compared to those in the normal weight BMI trajectory, girls in early onset obesity trajectories had an increased risk of elevated BP with odds ratio (OR) of 2.18 (95% confidence interval 1.31 to 4.20) and 1.95 (1.01 to 3.77). We also observed the similar associations, though not significant, for boys in early onset overweight trajectory, (p-value = 0.18 and odds ratio of 2.39 (0.67 to 8.57)). Distinct weight trajectories are observed in black South African children from as early as five years. Early onset adiposity trajectories are associated with elevated BP in both boys and girls. It is important to consider individual patterns of early-life BMI development, so that intervention strategies can be targeted to at-risk individuals.

3.3 Introduction

Hypertension is a global public health problem affecting more than one billion people globally (Alwan, 2011b). It is a risk factor for developing cardiovascular disease (CVD) and also contributes to an increase in mortality worldwide. A recent systematic analysis reported that hypertension accounts for about nine million deaths globally every year (Lim et al., 2013) . In African countries, 25% of deaths are caused by hypertension (Alwan, 2011b). Hypertension is also one of the leading causes of heart attacks, stroke and kidney failure (Lim et al., 2013). Results from the Heart of Soweto Study reported a 55% hypertension prevalence among adult South Africans aged 52.8 years on average (Sliwa et al., 2008). Some studies have looked at elevated blood pressure (BP) in childhood, mostly in rural South Africa, with reported prevalence rates ranging from 1 to 25.9% (Daniel et al., 2013; Kemp et al., 2011; Monyeki et al., 2006; Schutte et al., 2003).

Previous studies have reported that childhood blood pressure, weight gain from childhood to adulthood, adulthood obesity (Bridger, 2009; Christofaro et al., 2011), childhood and adolescent physical in-activity and certain lifestyle behaviors such as use of tobacco and alcohol consumption (Petkeviciene et al., 2014), are some of the determinant factors of adulthood hypertension. Understanding the life-course progression of adiposity in children is important since childhood adiposity is associated with adult obesity which has been reported to be linked to increased hypertension risk in adults (Singh et al., 2008). Kagura and colleagues reported that elevated BP can be observed from childhood in black urban South African children (Kagura et al., 2015). Most of the studies that reported on childhood obesity and adult hypertension have used cross sectional data. There are few studies that have used longitudinal data to understand the effect of life-course childhood adiposity on late adolescent blood pressure and lack of comprehensive longitudinal data have made it difficult to do such studies in an African setting.

There has been increased interest in the role of growth patterns on BP, it is also still unclear how early childhood adiposity influences later BP (Jones et al., 2012). Using longitudinal data will help elucidate this relationship and this study in particular will add further evidence from an African perspective. For the first time in an exclusively African population, we sought to determine whether adiposity progression (from 5 to 18 years of age), focused on using body mass index (BMI) as an adiposity marker, could be used to predict BP among late adolescents.

We use a relatively new method in both biology and epidemiology called Latent Class Growth Mixture Modeling (LCGMM) to identify distinct sex-specific adiposity trajectories. LCGMM groups

individuals with the same developmental trajectory in the same class. It also allows for inclusion of variation in several growth parameters both within and between classes (Berlin, Parra, et al., 2014a). Developmental trajectories of BMI describe individuals' BMI change over time. One of the advantages for using LCGMM is that adiposity trajectories are identified independently without pre-assumptions of the relative contributions of various lifestyles, genetic and epigenetic factors. Therefore multiple factors may contribute to variation in the information of these adiposity trajectories.

The aims of this study were to: 1) determine the distinct sex-specific patterns of adiposity trajectories in black South Africa children from 5 to 18 years of age, 2) find the prevalence of elevated blood pressure in late adolescence, and 3) to explore associations between these distinct adiposity trajectories to elevated BP in late adolescence.

3.4 Methods

3.4.1 Participants

To identify developmental patterns of BMI from 5 to 18 years old and relate them to blood pressure in late adolescence, data from the Birth to Twenty Plus cohort (Bt20+) were used. Bt20+ is Africa's largest and longest running longitudinal birth cohort, with 3273 children at time of enrolment. It is focused on the health and development of children born in a South African urban township, namely Soweto in Johannesburg. The cohort comprised of 1682 girls and 1591 boys of which the majority were black children (78.4%). The participants have been followed up to date using different means of communication. The detailed cohort profile with recruitment and cohort attrition details has been described elsewhere (Richter et al., 2007). Only black participants (n=1824) with weight and height data available for at least two time points between 5 to 18 years were included in this study.

3.4.2 Measures

Anthropometrics

Trained research assistants collected anthropometric measurements. Weight was measured using a digital scale to the nearest 0.1 kg with participants in light clothes and without shoes. A wall-mounted stadiometer (Holtain, UK) was used to measure standing height to the nearest 0.1 cm throughout the cohort. Weight and height at 5 years and 7-18 years old were used to calculate BMI (weight (kg)/height (m^2)), used as a marker for adiposity at corresponding years.

Blood pressure assessment

Blood pressure (mm Hg) was measured using Omron 6 automated machine (Kyoto, Japan) from 8 years of age onwards and a Dinamap Vital Signs monitor 1846SX (Critikon, USA) was used at 5 years of age. At each assessment, participants' seated blood pressure was measured three times with a 2 min interval between each measurement. The blood pressure measurements were taken after 5 min of seated rest. The mean average for the second and third right arm readings was recorded for the current analysis. Blood pressure measurements from one visit have been used in blood pressure studies before (Abolfotouh et al., 2011; Afrifa–Anane et al., 2015; Jones et al., 2012; Kagura et al., 2015).

The mean arterial pressure (MAP) was calculated from systolic blood pressure (SBP) and diastolic blood pressure (DBP) using the formula; MAP= (SBP+ (2*DBP))/3. We followed a standard procedure as recommended by the fourth National High Blood Pressure Education Program working group on high blood pressure in children and adolescents (NHBPEP) (Health et al., 2012) report to classify blood pressure either normotensive (BP less than 90th percentile), prehypertension or hypertension (BP equal or over 90th percentile for sex, age and height) for those participants who were above 17 years but not yet 18 years old the time BP data was collected. For participants who were already 18 years or older we used the cut-offs as recommended in the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (Chobanian, 2003). Prehypertension was defined as SBP readings from 120 to 139 mm Hg or a DBP from 80 to 89 mm Hg while hypertensive was defined as SBP readings equal or greater than 140 mm Hg or DBP readings equal or greater than 90 mm Hg. Due to small sample size the prevalence of hypertension, prehypertension and hypertension were combined and defined as elevated blood pressure

3.4.3 Statistical Analysis

Different group based modeling methods such as Latent Class Growth Analysis (LCGA) and Latent Class Growth Mixture Modeling (LCGMM), have been used to identify and classify individuals into different trajectories. LCGA technique was developed by Nagin and colleagues (Hennig et al., 2009; Kelly et al., 2008; Nagin, 2005; Puoane et al., 2002) and is implemented through SAS Proc Traj in the SAS software. There is also a Traj Stata software plugin developed by Jones and Nagin, 2012 (Rossouw et al., 2012b) . On the other hand LCGMM was developed by Muthén and colleagues and is implemented in Mplus (Mei et al., 2012; Muthen, 2001; Muthén, 2006; Muthén, 1998-2012). We used Mplus and LCGMM since it allows variation in the intercept and slope in both within the class

(inter-individual differences) and across classes while SAS Proc Traj allows only across classes variation. Using LCGMM adds more heterogeneity in the model, thus being the more flexible method as such was preferred in the current analysis (Berlin, Parra, et al., 2014a; Hoekstra et al., 2011; Jung et al., 2008; Kelly et al., 2008; Muthen, 2001; Muthén, 2006; Muthén, 1998-2012).

The exploratory analyses to check the patterns of missing data concluded that data was missing at random. Mplus handles missing data by the expectation-maximization algorithm (EM algorithm) with assumption of missing at random (MAR) (Muthén, 1998-2012). This makes use of full information maximum likelihood estimation with missing values (FIML) by using estimation to integrate all available information based on MAR assumptions. This means that we can make full use of all available data in our analysis. This prevents the inclusion of only those participants who have no missing data at all data points, which would subsequently reduce the sample size. So the use of the maximum likelihood (ML) approach implemented in Mplus overcomes potential biases in participants with substantial missing data (Enders et al., 2001; Muthen, 2001; Muthén, 2006; Muthén, 1998-2012; Steyn et al., 2007).

We included only those participants with at least two time points of available data for BMI in the current analysis. Those with one time point of available data were excluded as this may have influenced the identification and pattern of the BMI trajectories. On average, seven available data points per participant were used to perform this longitudinal analysis.

Latent Class Growth mixture modeling (LCGMM in Mplus version 7.3 (Muthén, 1998-2012) was performed to identify the distinct sex-specific BMI trajectories between 5 to 18 years.
The BMI values were used in this study because they are more sensitive to changes in body composition and have been recommended over BMI z-scores (Berkey et al., 2007; Boyer et al., 2015; Cole et al., 2005).
Once BMI trajectory group membership was determined for both girls and boys, each respondent was assigned to the class according to the highest probability of belonging to that class. The remainder of analyses was conducted using Stata version 13 (StataCorp, 2013) to examine additional descriptive characteristics of the classes. We used both standard logistic and multivariate linear regressions to estimate the relationship between latent class membership and blood pressure in late adolescence. Analysis of variance (ANOVA) and chi-square test results were used to assess the differences in different study characteristics among the BMI trajectory classes at 5% level of significance.

3.4.4 Ethical considerations

Prior to the study, ethical approval was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg (Reference number M0101556), participants or their parents/caregivers when the participants were minor gave informed consent at the beginning of each data collection session throughout the study.

3.5 Results

3.5.1 BMI Trajectories: optimal number of BMI trajectories

Using LCGMM, we explored linear, quadratic and cubic slopes to fit the model. The cubic slope coefficients were either not significant or required prohibitive running times and did not converge in all models. This suggests that the cubic time function model did not fit the data well. After applying the criteria as recommended by other authors (Berlin, Parra, et al., 2014a; Dahly, 2012; Hoekstra et al., 2011; Jung et al., 2008; Muthen, 2001; Muthén, 2006; Ram et al., 2009; Wang et al., 2007), only quadratic models involving quadratic time function were further analyzed.

To determine the optimal number of latent classes, we used different model fit indices in conjunction with other criteria as used in previous studies(Berlin, Parra, et al., 2014a; Dahly, 2012; Jung et al., 2008; Magee et al., 2013; Tofighi et al., 2008). Firstly, we looked at the three model statistics, the Bayesian Information Criterion (BIC) (Nylund et al., 2007), the Bootstrap Likelihood Ratio Test (BLRT) (McLachlan et al., 2004) and Lo, Mendell and Rubin Likelihood Ratio Test (LMR-LRT) (Lo et al., 2001). Some studies have applied BIC in variable modeling analyses (Garden et al., 2012; Magee et al., 2013; Nylund et al., 2007; Ventura et al., 2009). It estimates a model to be true using posterior probabilities. A lower BIC would indicate that a model is more likely to be considered as the true model (Nylund et al., 2007). BIC reduces the false positive rate, hence most individuals will be assigned in their right BMI latent class (Dziak et al., 2012). We also looked at the entropy value and number of study participants per class. We employed a less restrictive 1% group membership in this study, which has also been used before in other studies (Jun et al., 2012; Magee et al., 2013). Lastly we looked at the shape of the BMI trajectories and its clinical interpretability.

Model fit statistics used to come up with optimal number of classes in both boys and girls are shown in Table 3-1. Applying the criterion discussed above a four-class model fits our data well in girls and a three-class solution has the best classification of BMI trajectory membership in boys.

In order to facilitate interpretation of the results, Figure 3-1 shows the BMI trajectory classes for 947 girls and 877 boys in relation to the extended International Obesity Task Force (IOFT) (Cole et al.,

2012). For girls, four BMI trajectory classes can be described as: Trajectory 1 (normal weight: 75.7%), Trajectory 2 (late onset overweight: 15%), Trajectory 3 (early onset obesity to overweight: 4.8%) and lastly Trajectory 4 (early onset obesity to morbidly obese: 4.5%). For boys, a three-class model fits the data well. These classes can be classified as: Trajectory 1 (normal weight: 92.7%), Trajectory 2 (early onset overweight to normal: 6%) and Trajectory 3 (the early onset overweight to obese trajectory: 1.3%)

Table 3-1: Model fit statistics for quadratic LCGMM comparing solutions for 1 to 7 latent classes

Sex	Latent classes	BIC[1]	LMR-LRT[2] (P-value)	BLRT[3] (P-value)	Convergenc	Entropy value	Log Likelihood	Number of parameters
Boys	1	23605.4	N/A	N/A	Yes	N/A	-11738.3	19
	2	22846.6	0.03	0.00	Yes	0.98	-11345.4	23
	***3**	**22644.4**	**0.13**	**0.67**	**Yes**	**0.99**	**-11230.7**	**27**
	4	22544.7	0.53	1.00	Yes	0.98	-11167.3	31
	5	22476.5	0.77	0.67	Yes	0.93	-11119.7	35
	6	22438.4	0.61	1.00	Yes	0.94	-11087.1	39
	7				NO			43
Girls	1	27949.8	N/A	N/A	Yes	N/A	-13909.8	19
	2	27226.2	0.00	0.00	Yes	0.94	-13534.3	23
	3	27155.0	0.10	0.00	Yes	0.76	-13485.0	27
	***4**	**27083.9**	**0.23**	**0.00**	**Yes**	**0.80**	**-13435.7**	**31**
	5	27026.0	0.45	0.6	Yes	0.82	-13393.1	35
	6	26990.9	0.63	1.00	Yes	0.78	-13361.8	39
	7	26961.2	0.27	1.00	Yes	0.80	-13333.3	43

[1] Bayesian Information Criterion,

[2] Lo, Mendell and Rubin Likelihood Ratio Test,

[3] Bootstrapped Likelihood Ratio Test, N/A – not applicable

* The optimal class solution according to the model fit criteria

LCGMM = Latent Class Growth Mixture Modeling

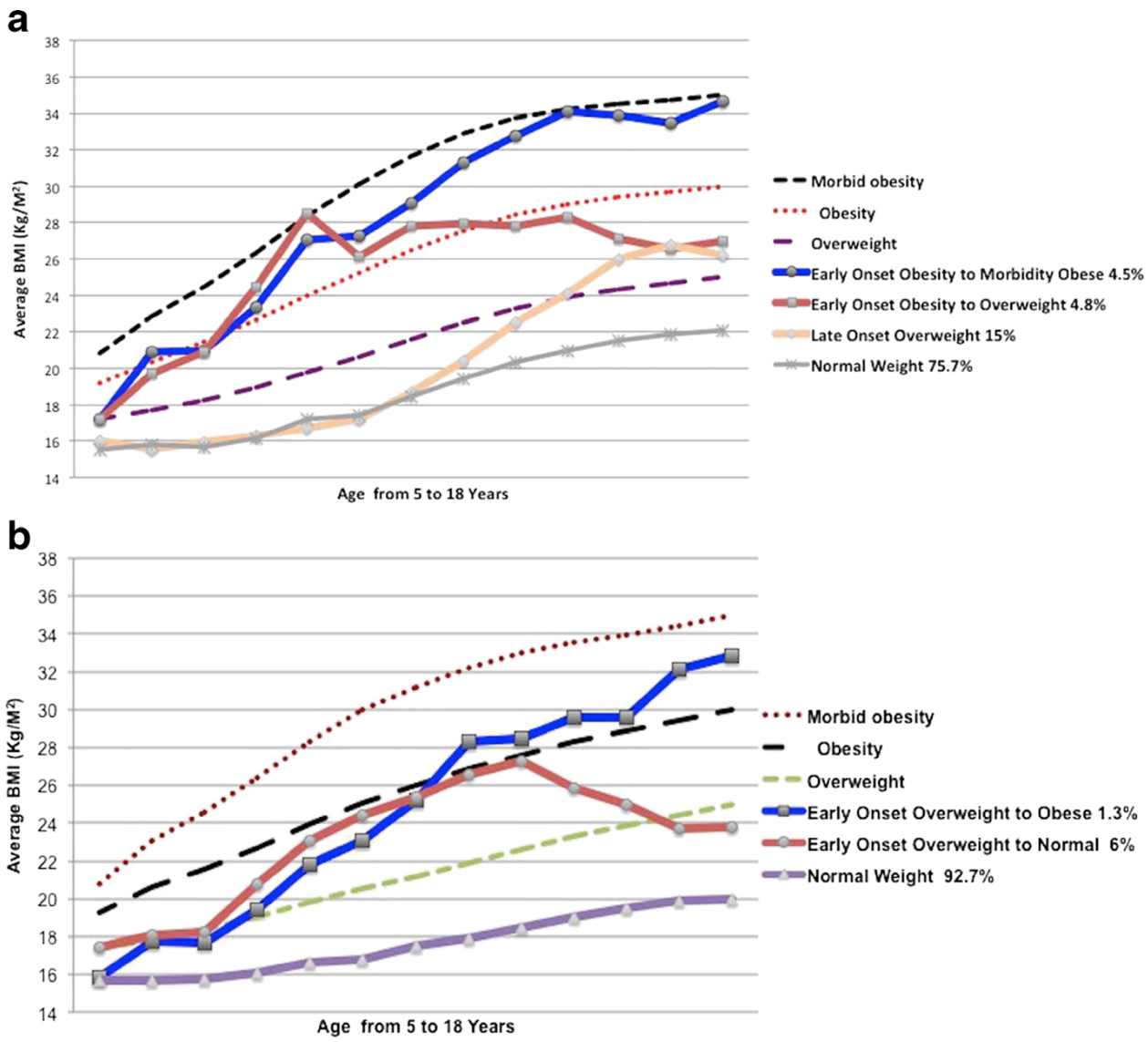

Figure 3-8: a BMI Trajectories in girls. BMI latent classes plotted together with Extended International Obesity Task Force (IOTF) Cut-Offs for BMI in girls. **b** BMI Trajectories in boys. BMI latent classes plotted together with Extended International Obesity Task Force (IOTF) Cut-Offs for BMI in boys.

3.5.2 Study characteristics

Study characteristics by BMI trajectory classes are shown in Tables 3-2 and 3-3. Most of the study characteristics were significantly different among BMI trajectories in girls. In boys, lower maternal education and SBP at 18 years were significantly different among the BMI trajectories and the rest were not.

Table 3-2: Study characteristics by BMI trajectory in girls: The Birth to Twenty Plus Cohort

	Normal weight (n=717)	**Trajectory 2 (n=142)**	**Trajectory 3 (n=45)**	**Trajectory 4 (n=43)**	**P value**
Study characteristics					
Birth weight (kg)	3.0 (0.5)	3.0 (0.5)	3.0 (0.5)	3.3 (0.5)	0.01*
Height at 18 years (cm)	159.7 (6.2)	158.4 (5.1)	157.3 (6.6)	159.3 (6.1)	0.03*
SBP at 18 years (mm Hg)	114.3 (9.6)	116.0 (9.5)	117.9 (11.7)	120.1 (11.3)	0.00***
DBP at 18 years (mm Hg)	71.5 (8.3)	72.8 (8.1)	75.8 (12.7)	75.8 (9.0)	0.00***
MAP at 18 years (mm Hg)	85.8 (7.9)	87.2 (7.8)	89.8 (11.8)	90.6 (8.9)	0.00***
Elevated BP n (%)	187 (28.5)	28 (31.1)	17 (43.6)	18 (46.1)	0.03*

For girls: Trajectory 2 = Late Onset Overweight, Trajectory 3 = Early Onset Obese to Overweight, Trajectory 4 = Early Onset Obese to Morbidity Obese.

Summary statistics presented as mean (±standard deviation) otherwise stated

* P<0.05; ** P< 0.01; ***P< 0.001

ANOVA and chi square test were conducted to describe the study characteristics differences in BMI trajectories

Percentages may not add up to 100% due to rounding.

Table 3-3: Study characteristics by BMI trajectory in boys: The Birth to Twenty Plus Cohort

	Normal weight (n=813)	**Trajectory 2 (n=53)**	**Trajectory 3 (n=11)**	**P value**
Study characteristics				
Birth weight (kg)	3.1 (0.5)	3.2 (0.6)	3.2 (0 .7)	0.46
Height at 18 years (cm)	170.6 (6.7)	172.4 (5.5)	170.4 (7.6)	0.21
SBP at 18 years (mm Hg)	121.0 (10.8)	124.2 (8.2)	134.5 (16.9)	0.00***
DBP at 18 years (mm Hg)	71.0 (8.6)	71.9 (8.3)	76.8 (8.7)	0.09
MAP at 18 years (mm Hg)	87.7 (8.1)	89.4 (7.3)	96.0 (10.3)	0.00**
Elevated BP n (%)	276 (38.7)	20 (46.5)	6 (60.0)	0.24

For boys: Trajectory 2 = Early Onset Overweight to Normal Weight, Trajectory 3 = Early Onset Overweight to Obese.

Summary statistics presented as mean (±standard deviation) otherwise stated

* P<0.05; ** P< 0.01; ***P< 0.001

ANOVA and chi square test were conducted to describe the study characteristics differences in BMI trajectories

Percentages may not add up to 100% due to rounding.

3.5.3 Blood pressure in late adolescence and BMI trajectory classes

Only participants with blood pressure values at 18 years of age were included in this analysis. It included 766 boys and 823 girls. The prevalence of elevated BP at 18 years was significantly higher in boys than girls (Chi2 = 14.33, P = 0.00). The overall prevalence of elevated blood pressure was 34.91% (39.43% boys and 30.38% girls). Birth weight and height at 18 years were added in the model to determine whether BMI trajectory classes have an effect on SBP, DBP, MAP and elevated BP status as shown in Table 3-4 In girls, Trajectory 3 was significantly associated with SBP, DBP and MAP with mean BP (95% confidence interval, 95%CI) of 4.18 mm Hg (1.03-7.33), 4.44 mm Hg (1.65 to 7.22) and 4.35 mm Hg (1.70 to 7.01) compared to normal trajectory, respectively. Trajectory 4 was significantly associated with SBP, DBP and MAP with mean BP of 6.08 mm Hg (2.93 to 9.23), 4.28 mm Hg (1.50 to 7.07) and 4.88mm Hg (2.23 to 7.54) compared to normal weight trajectory, accordingly. Girls in Trajectories 3 and 4 had a 1.95 fold (1.01 to 3.77) and a 2.18 fold (1.31 to 4.20) increased risk of elevated BP in late adolescence, separately.

For boys, Trajectory 3 was significantly associated with SBP, DBP and MAP with mean elevated BP of 13.45 mm Hg (6.82 to 20.07), 5.74 mm Hg (0.30 to 11.10) and 8.31 mm Hg (3.30-13.32) compared to normal weight trajectory respectively. In boys we observed a weak association for Trajectory 3 with a 2.39 fold (0.67 to 8.57) increased risk of elevated BP in late adolescence.

The main results did not vary adjusting by birth weight, height at 18 years or both in girls. In boys, only the association between Trajectory 3 and DBP varied after adjusting by both weight and height at 18 years. The association was not significant in the unadjusted model while significant in the adjusted model.

Table 3-4: The Association of BMI Trajectories, Birth Weight and Height and Blood Pressure at 18 years for girls

	Unadjusted	**Adjusted**
SBP (mm Hg)		
BMI Trajectory		
Normal Weight	Reference	Reference
Late Onset Overweight	1.77 (-0.38 to 3.93)[1]	2.07 (-0.08 to 4.21)
Early Onset Obese to Overweight	3.66 (0.50 to 6.82)*	4.18 (1.03 to 7.33)**
Early Onset Obese to Morbidity		
Obese	5.87 (2.71 to 9.03)**	6.08 (2.93 to 9.23)**
Birth Weight (kg)		-0.61 (-1.99 to 0.78)
Height (cm) at 18 years		0.22 (0.11 to 0.34)**
R^2	0.02	0.04
DBP (mm Hg)		
BMI Trajectory		
Normal Weight	Reference	Reference
Late Onset Overweight	1.33 (-0.57 to 3.22)	1.40 (-0.50 to 3.30)
Early Onset Obese to Overweight	4.29 (1.52 to 7.07)**	4.44 (1.65 to 7.22)**
Early Onset Obese to Morbidity		
Obese	4.25 (1.48 to 7.03)**	4.28 (1.50 to 7.07)**
Birth Weight (kg)		-0.05 (-1.27 to 1.18)
Height (cm) at 18 years		0.06 (-0.04 to 0.16)
R^2	0.02	0.02

* P<0.05; ** P< 0.01; *** P< 0.001. [1]Intercept (SBP, DBP) and 95% Confidence Interval are presented.

SBP= systolic blood pressure, DBP = diastolic blood pressure. Adjusted for birth weight and height at 18 years.

Table 3-4: The Association of BMI Trajectories, Birth Weight and Height and Blood Pressure at 18 years for girls ….cont

	Unadjusted	**Adjusted**
MAP (mm Hg)		
BMI Trajectory		
Normal Weight	Reference	Reference
Late Onset Overweight	1.47 (-0.33 to 3.28) [1]	1.62 (-0.19 to 3.43)
Early Onset Obese to Overweight	4.08 (1.43 to 6.73)***	4.35 (1.70 to 7.01)***
Early Onset Obese to Morbidity Obese	4.79 (2.14 to 7.44)***	4.88 (2.23 to 7.54)***
Birth Weight (kg)		-0.23 (-1.40 to 0.93)
Height (cm) at 18 years		0.12 (0.02 to 0.21)*
R^2	0.03	0.03
Elevated BP at 18 years (Normal BP-reference)	OR	OR
BMI Trajectory		
Normal Weight	Reference	Reference
Late Onset Overweight	1.13 (0.70 to 1.82)	1.14 (0.71 to 1.84)
Early Onset Obese to Overweight	1.93 (1.00 to 3.72)*	1.95 (1.01 to 3.77)*
Early Onset Obese to Morbidity Obese	2.15 (1.11 to 4.12)*	2.18 (1.31 to 4.20)*
Birth Weight (kg)		0.93 (0.67 to 1.27)
Height (cm) at 18 years		1.01 (0.98 to 1.03)
Pseudo R^2	0.01	0.01

* P<0.05; ** P< 0.01; *** P< 0.001.

Adjusted for birth weight and height at 18 years.

[1]Intercept (MAP), OR (elevated BP) and 95% Confidence Interval are presented.

MAP = mean arterial pressure; OR = Odds Ratio

Table 3-5: The Association of BMI Trajectories, Birth Weight and Height and Blood Pressure at 18 years for boys

	Unadjusted	**Adjusted**
SBP (mm Hg)		
BMI Trajectory		
Normal Weight	Reference	Reference
Early Onset Overweight to Normal Weight	3.23 (-0.08 to 6.54)[1]	2.85 (-0.43 to 6.13)
Early Onset Overweight to Obese	13.46 (6.75 to 20.17)**	13.45 (6.82 to 20.07)**
Birth Weight (kg)		-1.35 (-2.85 to 0.14)
Height (cm) at 18 years		0.27 (0.15 to 0.38)**
R^2	0.02	0.05
DBP (mm Hg)		
BMI Trajectory		
Normal Weight	Reference	Reference
Early Onset Overweight to Normal Weight	0.95 (-1.69 to 3.59)	0.844 (-1.80 to 3.49)
Early Onset Overweight to Obese	5.77 (0.41 to 11.13)	5.74 (0.39 to 11.10)*
Birth Weight (kg)		-0.53 (-1.7 to 0.68)
Height (cm) at 18 years		0.07 (-0.2 to 0.17)
R^2	0.01	0.01

* P<0.05; ** P< 0.01; *** P< 0.001

Adjusted for birth weight and height at 18 years

[1]Intercept (SBP, DBP) and 95% Confidence Interval are presented.

SBP= systolic blood pressure, DBP = diastolic blood pressure

Table 3-5: The Association of BMI Trajectories, Birth Weight and Height and Blood Pressure at 18 for boys...cont

	Unadjusted	**Adjusted**
MAP (mm Hg)		
BMI Trajectory		
Normal Weight	Reference	Reference
Early Onset Overweight to Normal Weight	1.71 (-0.77 to 4.19)[1]	1.51 (-0.96 to 3.99)
Early Onset Overweight to Obese	8.33 (3.3 to 13.37)***	8.31 (3.30 to 13.32)***
Birth Weight (kg)		-0.80 (-1.93 to 0.33)
Height (cm) at 18 years		0.14 (0.05 to 0.22)**
R^2	0.02	0.03
Elevated BP at 18 years (Normal BP-reference)	OR	OR
BMI Trajectory		
Normal Weight	Reference	Reference
Early Onset Overweight to Normal Weight	1.38 (0.74 to 2.55)	1.34(0.72 to 2.50)
Early Onset Overweight to Obese	2.38 (0.66 to 8.49)	2.39 (0.67 to 8.57)
Birth Weight (kg)		0.89 (0.66 to 1.18)
Height (cm) at 18 years		1.02 (1.00 to 1.05)*
Pseudo R^2	0.003	0.01

* P<0.05; ** P< 0.01; ***P< 0.001

Adjusted for birth weight and height at 18 years

[1]Intercept (MAP), OR (elevated BP) and 95% Confidence Interval are presented.

MAP = mean arterial pressure; OR = Odds Ratio

3.6 Discussion

There has been a growing interest in studying the heterogeneity in adiposity (BMI) developmental patterns in different populations over past years. Taking advantage of data-driven methods such as LCGMM we explored the distinct developmental patterns of BMI across childhood to late adolescence and its association with late adolescent blood pressure in black South African children. Our results confirm that there is heterogeneity in BMI trajectories in the study sample and that trajectories vary between boys and girls. A four-class BMI trajectory model may best represent heterogeneity in BMI developmental patterns in girls (normal weight, late onset overweight, early onset obesity to overweight and early onset obesity to morbidly obese) and a three–class model among boys (normal weight, early onset overweight to normal and early onset overweight to obese trajectory). These results are consistent with those reported by Hejazi and colleagues in Canadian children aged 2-8 years but with different age group and ancestry population (Hejazi et al., 2009). Similarly, a study by Haga et al reported that BMI trajectories varied between boys and girls in Japanese children aged between 0 to 12 years (Haga et al., 2012). Ventura et al. (Ventura et al., 2009) reported four distinct BMI trajectories in girls only. Few studies have focused on gender differences in BMI trajectories. American, Canadian, Australian and Dutch studies in children reported either three or four-class distinct BMI trajectories in both girls and boys (Brault et al., 2015; Garden et al., 2012; Hoekstra et al., 2011; Jun et al., 2011; Li et al., 2007; Magee et al., 2013; Mustillo et al., 2003; Nonnemaker et al., 2009; Pryor et al., 2011). Individuals in trajectories characterized by high BMI trends had an increased risk of developing hypertension in late adolescence compared to those in a normal weight trajectory in the current study.

Prevalence of elevated BP in late adolescence was relatively higher in boys (39.43%) than in girls (30.38%). This prevalence is consistent with a recent study in urban South African children that reported the prevalence of prehypertension or hypertension ranging between 17.6 to 40.8% (Kagura et al., 2015). Another study in youth aged 15 to 24 years in rural Ghana reported levels of prehypertension or hypertension with a prevalence of 37.4%. The prevalence of prehypertension or hypertension (48.8 % vs. 26.6 %) was higher in males than in females (Afrifa–Anane et al., 2015). It should be pointed out that the prevalence in the current study was also higher than that previously reported in some studies within and outside South Africa. A study done by Makgae and colleagues in rural South African children aged 6 to 13 years found that 1.0 to 5.8% of boys and 3.1 to 11.4% of girls were hypertensive (Makgae et al., 2007). Moselakgomo et al. assessed BMI and blood pressure among adolescent school children aged 10 to16 years in Limpopo province, South Africa and the

results revealed that prevalence of hypertension was between 2.3 to 5.9% (Moselakgomo et al., 2012). The following blood pressure prevalence has been reported in other African countries. In Brazzaville, Congo 24.3% in girls and 16.6% prevalence of hypertension in boys school children aged 5 to 18 years (Ellenga Mbolla et al., 2014). In Egyptian adolescents aged 11-19 years revealed that the prevalence rates of prehypertension and hypertension were 5.7% and 4.0%, respectively (Abolfotouh et al., 2011). The mechanisms of the observed sex differences in elevated BP in our study sample are not clear but may be attributed to sexual dimorphism as observed in other BP control programs (Maranon et al., 2013) and also due to cardiovascular fetal programming as reported before (Maranon et al., 2013)·(Kautzky‐Willer et al., 2009).

Girls in the early onset obesity trajectories were likely to have elevated SBP, DBP and MAP in late adolescence compared to those in a healthy trajectory. They displayed a 118% increased odds of developing hypertension in late adolescence compared to those in the normal weight trajectory. Boys in early onset overweight to obese trajectory were likely to have a higher SBP, DBP and MAP in late adolescence. For instance boys in this trajectory had a 13 mm Hg mean SBP higher than those in a normal weight trajectory.

One longitudinal study on life-course adiposity and blood pressure in late adolescents has been done in Australian children (Huang et al., 2015), three of the seven adiposity trajectories were associated with elevated blood pressure at 17 years old of age. We report that being in an early onset obese or overweight trajectory was associated with increased risk of elevated BP in both girls and boys which is in agreement with other studies, although comparative studies were performed in a different population and different age range (Huang et al., 2015). Girls in the early onset obesity to overweight BMI trajectory displaced a reduced risk of elevated BP in late adolescence compared to those who were in early onset obesity to morbidity obese trajectory, though the CIs do overlap, despite the fact that both groups had early onset obesity. This study was not an intervention study but it is important to study the mechanisms behind the weight loss in this group of girls. This could be important in reducing hypertension risk later in life.

The current study has several strengths; firstly, the longitudinal study in black South African children is a representative sample of South African children hence more relevant in understanding the different BMI trajectories in black South African children. Using LCGMM in identifying trajectories is an ideal analytical method since it involves a number of criteria in selecting the best fitting model and it predicts individual class membership from the available data and its ability to deal with missing

at random data. The current results therefore clearly show individual heterogeneity in BMI development in boys and girls from five years to late adolescence. Our analysis was stratified according to sex and performed in a South African black population, which gives us an in-depth understanding of the difference in BMI developmental patterns in black boys and girls in South Africa. These results suggest that targeted intervention might be developed for individuals in high-risk trajectories.

Limitations of our current study include the fact that the sample used in this study is from Soweto, South Africa and generalizability to other parts of Africa should be treated with caution. Adiposity trajectories explain only 5% variation in late adolescent blood pressure and we do not know yet what the other factors are, that would explain the remaining variance. We speculate that the role of genetics and epigenetics could be important, as previously reported (Ehret, Munroe, et al., 2011). The role of genetics and epigenetics on adiposity and growth patterns in African populations is understudied and it calls for further investigation. A recent study by Kagura and colleagues in this cohort reported no association between blood pressure at 18 and alcohol consumption or smoking or both at 18 years in this population (Silva et al., 2013) and thus we did not include these variables within the models. Furthermore, we examined walking to school as a proxy of physical activity but this variable was not able to different among participants given that most participants are walking extensively during the day to and from school.

Due to the limited sample size we might not be able to capture all trajectories; this also influenced the observation that the high-risk trajectory in boys comprised of very few individuals that might influence association analysis. Potential bias cannot be ruled out, because we had some lost to follow up at some years in this study

3.7 Conclusions

In this study, we identified distinct sex-specific trajectories. The early onset obesity or overweight trajectories are associated with elevated blood pressure in late adolescence. These results signify the importance to consider patterns of BMI development, especially at early stage of development, so that prevention strategies may be implemented to target those individuals in high-risk developmental patterns.

Our study has shown that patterns of adiposity could be a preferred predictor of future SBP, DBP, MAP and elevated BP in late adolescents compared to cross-sectional BMI measures. Furthermore, identification of childhood obesity can help with early identification of those at risk of developing elevated BP and other chronic diseases in adulthood.

3.8 References

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Chapter 4

Early life growth predictors of childhood adiposity trajectories and future risk for obesity: Birth to Twenty Plus Cohort

Published online ahead of print: ***Childhood Obesity; doi: 10.1089/chi.2016.0310***

Chapter 4: Early life growth predictors of childhood adiposity trajectories and future risk for obesity: Birth to Twenty Plus Cohort

4.1 Preface – Empirical Paper 2

This chapter was published as a second empirical research paper as follows. Munthali RJ, Kagura J, Lombard Z, Norris SA. Early Life Growth Predictors of Childhood Adiposity Trajectories and Future Risk for Obesity: Birth to Twenty Plus Cohort. ***Childhood Obesity; doi: 10.1089/chi.2016.0310***

The manuscript presents the second part of the study investigating the early life growth factors associated with particular BMI trajectory membership and explores the associated risk of overweight or obesity in young adulthood. It follows up on the findings of the first empirical chapter, which identified variation in BMI developmental patterns in black South African children. It identified three and four distinct BMI trajectories in boys and girls respectively; these trajectories were associated with elevated blood pressure in late adolescence. This study helps to identify early life factors that put individuals in or facilitate transitioning into an obese or overweight trajectory, which exposes them to high risk of the developing obesity and related metabolic disease risk, such as BP, later in life. We utilized the already established BMI trajectories from the first manuscript and data on early life growth factors such as birth weight, relative conditional relative weight gain before five years of age, maternal factors and social economic status in black South African children from the Birth to Twenty Cohort Plus.

The individual contributions by the candidate and any co-authors to the work are as follow: R.J. M, J.K, Z.L and S.A.N carried out the initial conceptualization and design of the study. RM led the study, conducted the initial analyses and interpreted the data, drafted the initial manuscript, and approved the final manuscript as submitted in close collaboration with J.K, Z.L and S.A.N who gave guidance throughout the study from conception to the approval of the final draft, reviewed and edited the data analysis plan and the manuscript for intellectual content. All authors read and approved the final manuscript as submitted.

4.2 Abstract

There is growing evidence of variations in adiposity trajectories among individuals, but the influence of early life growth patterns on these trajectories is under-researched in low- and middle-income countries. Therefore, our aim was to examine the association between early life conditional relative weight gain and childhood adiposity trajectories. We previously identified distinct adiposity trajectories (four for girls and three for boys) in black South African children
(boys =877; girls = 947). The association between the trajectories and early life growth patterns and future obesity risk was assessed by multivariate linear and multinomial logistic and logistic regressions. Conditional relative weight gain independent of height was computed for infancy (0-2 years) and early childhood (2-4 years). Anthropometric measures at 23 years of age were used to assess risk for overweight in young adulthood. Conditional relative weight gain before five years of age was significantly associated with early-onset of obesity or overweight (excess weight) BMI trajectories in both boys and girls. In girls, greater conditional relative weight gain in infancy was associated with increased relative risk of being in the early-onset obese to morbid obese trajectory, with relative risk ratios (RRR) of 2.03 (95% confidence interval: 1.17-3.52) compared to belonging to a BMI trajectory in the normal range. Boys and girls in the early-onset obesity or overweight BMI trajectories were more likely to be overweight or obese in early adulthood. Excessive weight gain in infancy and early childhood, independent of linear growth, predicts childhood and adolescent BMI trajectories towards obesity. These results underscore the importance of early life factors in the development of obesity and other NCDs in later life.

4.3 Introduction

Obesity has become a worldwide growing public health burden (Anderson et al., 2006; Jones-Smith et al., 2013; Morris et al.; Ng et al., 2014; Sabin et al., 2015). Excess adiposity is associated with greater risk for chronic diseases such as type 2 diabetes mellitus (T2D), hypertension, and several types of cancer and increased mortality (Berrington de Gonzalez et al., 2010; Chen et al., 2012; Ellulu et al., 2014; Gupta et al., 2012; Zheng et al., 2013; Zheng et al., 2011). Understanding obesity trajectories requires longitudinal research that would accurately recognize individuals at higher risk of becoming overweight or obese, and more importantly identify critical periods of intervention.

There is growing evidence that studying weight gain across the life-course is essential in identifying early life determinants and future risk of obesity (Cimino et al., 2016; Cois et al., 2015; Haga et al., 2012; Howe et al., 2015; Huang et al., 2015; Monyeki et al., 2008). However, the majority of longitudinal studies related to weight gain and obesity from childhood to adulthood are from high-income countries (HICs), with a paucity of research focused on low- or middle-income countries (LMICs) where obesity rates are on the rise. Birth weight, both low and high, has widely been used as indicator of future risk of obesity and different non-

communicable diseases such as type 2 diabetes and stroke among others (Araújo et al., 2017; Whincup et al., 2008; Wilcox, 2001). Since birth weight is a proxy for intrauterine growth (De França et al., 2015), there is a need to understand the role of other early life factors, such as postnatal weight gain, on future risk of undesirable health outcomes.

Conditional growth measures, such as conditional relative weight and conditional height, have widely been employed to measure uncorrelated growth rate between repeated growth measures for the past decade (Adair et al., 2013; Adair et al., 2009; Keijzer-Veen et al., 2005; Li et al., 2003). Conditional relative weight shows deviation in weight of an individual from the expected size on the basis of their own previous measures and the growth of the other children in the study population (De Beer et al., 2015). Using early life conditional relative weight, we eliminate the influence of early length/height gain in predicting the future risk of obesity, since height gain has been reported to be protective to obesity (Menezes et al., 2011). Both conditional relative weight and conditional height at different growth periods have been reported to be associated with different cross-sectional body composition measures later in life (Adair et al., 2013; De Beer et al., 2015; De França et al., 2015), but the influence of early life conditional relative weight in infancy and early childhood on life-course BMI measures is not well established.

Data from the urban Birth to Twenty cohort (Bt20), Africa's longest running birth cohort study, reported that being overweight or obese in both infancy and early childhood was strongly associated with obesity in late adolescence (Lundeen et al., 2016).

To better understand the development of obesity in South African children, we previously employed modeling techniques that can identify BMI trajectories across childhood (age 5 years) to late adolescence (age 18 years). Through this technique we identified four adiposity trajectories for girls and three for boys in the Bt20 cohort (Munthali et al., 2016). These results underpin the existence of variation in childhood BMI development patterns, which were observed from as early as five years of age. It is therefore important to understand which early life factors may accelerate an individual's progression from a normal BMI trajectory pattern to one characterized with elevated BMI.

We hypothesized that early life growth factors such as birth weight and excessive postnatal weight gain are associated with adiposity trajectories from childhood to late adolescence. Therefore, in this study we endeavored to establish if early life conditional relative weight gain was associated with membership of specific BMI growth trajectories. Furthermore, we investigated if affiliation with a specific BMI trajectory would predict an increased risk of being excess weight in young adulthood at the age of 23 years.

4.4 Materials and Methods

4.4.1 Study sample

We analyzed data from the Bt20, a birth cohort of predominantly black South African (78.4 %) children (n=3273 at the time of enrolment), born in Soweto, Johannesburg, South Africa. The detailed cohort profile with recruitment and cohort attrition details has been described elsewhere (Richter et al., 2007). Only those participants with BMI trajectory data were included in the current study. Data on demographic and socio-economic variables were collected at birth or soon thereafter (Richter et al., 2012). Ethics approval for this work was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg (Reference number M0101556), participants or their caregivers provided written informed consent throughout the study.

4.4.2 Assessment of growth

Birth weight, weight and height at subsequent data collecting dates were collected by trained research assistants. Weight was measured using a digital scale to the nearest 0.1 kg with participants in light clothes and without shoes. A wall-mounted stadiometer (Holtain, UK) was used to measure standing height to the nearest 0.1 cm throughout the cohort. Weight and height at five years and 7 to 18 years of age were used to calculate BMI (weight (kg)/height (m^2)) at corresponding years.

We dichotomized BMI in early adulthood (at 23 years of age) as normal (BMI <25.0 kgm^{-2}) and overweight (BMI ≥25.0 kgm^{-2}) using the Extended international (International Obesity Task Force; IOTF) body mass index cut-offs (Cole et al., 2012). Due to a limited number of individuals being categorized as obese (BMI ≥30 kgm-2), we included those characterized as overweight and obesity as one group and defined it as excess weight. Birth weight and weight-for-age Z-scores were computed using the WHO growth standards (De Onis, 2006). Due to high correlation of repeated weight measures, conditional relative weight gain independent of height was calculated and used where appropriate. Conditional relative weight gain was calculated in infancy (0-2 years) and (2- 4 years with average age of five years) as standardized residuals (SR) from sex-specific linear regressions of current weight on prior weights, heights and current height. For instance, conditional relative weight gain at 2 years of age was calculated as SR from regressing weight at 2 on birth weight, birth length and height at 2 years old (Adair et al., 2013; Keijzer-Veen et al., 2005). A positive conditional relative weight gain value indicates growing faster than expected given prior size.

4.4.3 Confounders and covariates

We selected potential confounders based on prior knowledge in published literature and possible association with the outcome variable. These include mother's height (measured soon after birth of the child), gestational age at birth (preterm = 28-36 weeks, full term > 37 weeks), mother's age at delivery (categorized as <25, 25-

29, and >=30) and parity (categorized as two or less live births = 1; > two live births = 2). Physical assets owned in the participants' household were used as a proxy for socio-economic status index and have been recommended before (Feeley et al., 2013; Houweling et al., 2003; Jones et al., 2008). SES in this study is composite variable score that captures the family social class in the first 24 months of life, and was represented as the sum of household physical assets reported by the mother or caregiver (Balen et al., 2010). It was generated by summing the number of assets owned in the household amongst the following: television, car, washing machine, fridge, phone, radio, microwave, cell phone, DVD/Video, DSTV (cable channel), computer, internet, medical aid.

In boys 5 mothers had no education, 379 went up to primary school, 274 up to secondary and 72 had an education beyond secondary school. In girls 12 mothers had no education, 376 were educated up to primary school, 321 up to secondary and 61 had an education beyond secondary school. We further categorized maternal education into two categories – up to high school and beyond high school.

4.4.4 Childhood adiposity trajectories

Latent Class Growth mixture modeling (LCGMM) was previously used to identify four (girls) and three (boys) distinct BMI trajectories in the same group of Bt20 participants (Munthali et al., 2016), and were used as outcome variables in the current study. Trajectory group membership was previously reported in 1824 black South African children (boys = 877, girls = 947) with at least two age-point BMI measures between 5 to 18 years of age. On average, seven BMI age-point measures were available per participant. Each individual was assigned to the class where they had a high likelihood to belong. For girls, the four reported BMI trajectory classes were defined as; group 1 ("normal weight": 75.7%), group 2 ("early-onset obesity to morbidly obese": 4.5%), group 3 (" early-onset obesity to overweight": 4.8%) and lastly group 4 ("late onset overweight": 15%). For boys, three BMI trajectory classes were reported. These classes were: group 1 ("normal weight": 92.7%), group 2 ("the early-onset overweight to obese trajectory": 1.3%), and lastly group 3 ("early-onset overweight to normal": 6%) .The normal weight group was used as a reference for analysis in both boys and girls.

4.4.5 Statistical Analaysis

We used Stata version 13 (StataCorp, 2013) to examine additional descriptive characteristics of the trajectory classes. We assessed the association between class membership and other early life variables such as birth weight and conditional relative weight gain in infancy and early childhood, using multinomial logistic regression. Finally, we used both logistic and multivariate linear regressions to estimate the relationship between BMI latent class membership and excess weight in early adulthood at the age of 23 years. Analysis of variance (ANOVA) and chi-square test results were used to determine differences in various study characteristics among the BMI trajectory classes with a cut-off for significance set at 5%.

4.5 Results

4.5.1 Study characteristics

We compared maternal education, parity and weight status (categorical) in each BMI trajectory class using chi square tests and compared the means of continuous early life factors across BMI trajectories. In girls; maternal education, social economic status, birth weight and weight status in early adulthood (at 23 years of age) were significantly different at a 5% confidence level among the adiposity trajectories, Table 4-1. Girls from higher SES score households were likely to be assigned in the early-onset excess weight trajectories. Only maternal education, weight status in early adulthood and gestation period were significantly different in boys, Table 4-2. This means that the education level varied across the BMI trajectories with those that did not go beyond high school likely to be assigned in the early-onset excess weight trajectories in both young adult men and women. For weight status as expected those with excess weight in early adulthood were likely to belong to the early-onset excess weight trajectory membership.

The average BMI values from 5-18 years are presented in Supplementary Table 1. Average BMI for boys (20.4 kg/m^2) was lower than that of girls (23.3 kg/m^2) at 18 years. The life-course BMI-for-age prevalence for overweight from childhood (at age 5 years) to late adolescence (at 18 years) using IOFT cut offs showed a steady increase in prevalence in girls than in boys. At five years of age the prevalence of excess weight was 11.06% for boys and 13.58% for girls. At nine years of age the prevalence of excess weight for girls was twice higher than in boys, 15.3% and 8.8%, respectively. This trend continued up to in late adolescence, where at 18 years of age prevalence of excess weight was three times more in girls (33.8%) than boys (9.6%).

Table 4-1: Study characteristics by body mass index trajectory in women: The Birth to Twenty Plus Cohort

Study characteristics	Group 1 (Normal weight: n=755)	Group 2 (Early Onset Obese to Morbidity Obese: n= 42)	Group 3 (Early Onset Obese to Overweight: n=43)	Group 4 (Late Onset Overweight: n= 107)	P value
Maternal education n (%)[a]					
Up to secondary school	652 (86.4)	39 (92.9)	31 (72.1)	96 (89.7)	0.02*
≥ High school	103 (13.6)	3 (7.1)	12 (27.9)	11 (10.3)	
Parity /birth order n (%)					
≤ 2 children	521 (69.0)	32 (76.2)	30 (69.8)	71 (66.4)	0.71
> 2 children	234 (31.0)	10 (23.8)	13 (30.2)	36 (33.6)	
SES score	4.85 (2.05)	5.03 (1.79)	5.58 (1.50)	4.34 (1.92)	0.03*
Birth weight (kg)	3.0 (0.5)	3.3 (0.5)	3.0 (0.5)	3.0 (0.5)	0.01*
Birth weight (z score)	-0.55 (1.17)	0.01 (1.18)	-0.67 (1.09)	-0.53(1.13)	0.01*
Maternal height (cm)	158.6 (5.8)	160.7 (5.7)	158.1 (5.6)	159.0 (5.1)	0.13
Maternal age (years)	25.6 (6.2)	26.3 (5.3)	26.9 (6.7)	25.5 (6.4)	0.47
Gestational age (weeks)	37.8 (1.9)	38.3 (2.0)	38.2 (2.0)	37.8 (1.6)	0.54
Weight status at 22/23 n (%)					
Normal weight n (%)	299 (62.2)	1 (3.1)	5 (19.2)	20 (28.6)	0.000***
Excess weight n (%)	182 (37.8)	31 (96.9)	21 (80.8)	50 (71.4)	

Summary statistics presented as mean (±standard deviation) otherwise stated.

* P<0.05; ***P< 0.001.

ANOVA and chi square test were conducted to describe the study characteristics differences in BMI trajectories.

[a]Percentages may not add up to 100% due to rounding.

ANOVA, analysis of variance, SES, social economic status.

Table 4-2: Study characteristics by body mass index trajectory in men: The Birth to Twenty Plus Cohort

Study characteristics	Group 1 (Normal weight: n=816)	Group 2 (Early Onset Overweight to Obese: n=11)	Group 3 (Early Onset Overweight to Normal Weight: n=50)	P value
Maternal education n (%)[a]				
Up to secondary school	697 (85.4)	7 (63.6)	38 (76.0)	0.03*
≥ High school	119 (14.6)	4 (36.4)	12 (24.0)	
Parity /birth order n (%)				
≤ 2 children	540 (66.2)	7 (63.6)	36 (72.0)	0.69
> 2 children	276 (33.8)	4 (36.4)	14 (28.0)	
SES score	4.80 (2.07)	6.29 (1.70)	5.34 (1.98)	0.06
Birth weight (kg)	3.1 (0.5)	3.2 (0 .7)	3.2 (0.6)	0.46
Birth weight (z score)	-0.52(1.10)	-0.45 (1.64)	-0.34 (1.17)	0.52
Maternal height (cm)	158.5 (6.0)	160.6 (8.0)	158.7 (5.6)	0.54
Maternal age (years)	25.8 (6.3)	26.2(6.1)	25.3 (6.0)	0.99
Gestational age (weeks)	37.9 (1.8)	38.7 (2.3)	38.4 (1.4)	0.00***
Weight status at 22/23 n (%)				
Normal weight	496 (87.0)	2 (28.6)	11 (44.0)	0.000***
Excess weight	74 (13.0)	5 (71.4)	14 (56.0)	

Summary statistics presented as mean (±standard deviation) otherwise stated.

* P<0.05; ***P< 0.001.

ANOVA and chi square test were conducted to describe the study characteristics differences in BMI trajectories.

[a]Percentages may not add up to 100% due to rounding.

ANOVA, analysis of variance, SES, social economic status.

4.5.2 Predictors of membership within each trajectory

Compared with those in normal range weight trajectory, conditional relative weight gain in infancy (RRR=2.0, 95% confidence interval [CI]: 1.2 to 3.5) was significantly associated with the early-onset obesity to morbid obesity trajectory in girls. In addition conditional relative weight gain in early childhood was significantly associated (RRR=1.8, 95% CI: 1.09 to 3.0) with the early-onset obesity to overweight trajectory (Table 4-3). Similarly in boys, conditional relative weight gain independent of linear growth in infancy is significantly associated with early-onset overweight to normal weight (RRR=2.5, 95% CI: 1.52 to 4.1) and early-onset overweight to obesity (RRR =5.4, $p<0.01$) trajectories, compared to the normal weight (Table 4-4). Birth weight and maternal height were not associated with any of the BMI trajectory classes in both girls and boys.

4.5.3 Adult excess weight and BMI trajectory classes

The prevalence of excess weight in early adulthood at the age of 23 years was 46.6% in women and 15.5% in men. Women in the early-onset obese to morbidly obese trajectory had a 44.8 fold increased risk of excess weight compared to those in normal weight trajectory while men in early-onset overweight to obese trajectory had a 13.6 fold increased risk (Supplementary Table 2).

Table 4-3: Relative Risk Ratios (RRR) and 95% Confidence Intervals of the Predictors of BMI Trajectory Membership Compared With the "Normal" Group for Women

Trajectories	Unadjusted (RRR)	Adjusted (RRR)
Normal Weight	-	-
Early Onset Obese to Morbidity Obese	-	-
Birth Weight (kg)	2.61 (1.03-6.57)*	1.30 (0.41-4.06)
Relative weight gain 0-2 years (z-score)	2.11 (1.29-3.45)**	2.03 (1.17-3.52)*
Relative weight gain 2-4 years (z-score)	1.18 (0.76-1.82)	0.99 (0.61-1.59)
Mother height (cm)	1.07 (0.98-1.17)	1.06 (0.96-1.17)
Pseudo R^2	0.04	0.06
Early Onset Obese to Overweight	-	-
Birth Weight (kg)	1.17 (0.41-3.31)	0.74 (0.23-2.43)
Relative weight gain 0-2 years (z-score)	1.65 (0.94-2.89)	1.75 (0.97-3.17)
Relative weight gain 2-4 years (z-score)	1.51(0.92-2.46)	1.82 (1.09-3.04)*
Mother height (cm)	1.03 (0.93-1.13)	1.03 (0.93-1.14)
Pseudo R^2	0.04	0.06
Late Onset Overweight	-	-
Birth Weight (kg)	0.95 (0.55-1.63)	0.99 (0.52-1.87)
Relative weight gain 0-2 years (z-score)	1.04 (0.77-1.41)	1.02 (0.75-1.39)
Relative weight gain 2-4 years (z-score)	1.26 (0.97-1.63)	1.17 (0.90-1.54)
Mother height (cm)	1.03 (0.99-1.08)	1.03 (0.98-1.08)
Pseudo R^2	0.04	0.06

* P<0.05; ** P< 0.01.

Unadjusted = birth weight + relative weight gain 0-2 years + relative weight gain 2-4 years + mother height.

Adjusted= unadjusted + maternal education + social economic status + mother age + gestation age.

Table 4-4: Relative Risk Ratios (RRR) and 95% Confidence Intervals of the Predictors of BMI Trajectory Membership Compared With the "Normal" Group for Men

Trajectories (Boys)	Unadjusted (RRR)	Adjusted (RRR)
Normal Weight	-	-
Early Onset Overweight to Obese		
Birth Weight (kg)	0.36 (0.07-1.98)	0.52 (0.07-3.67)
Relative weight gain 0-2 years (z-score)	2.43 (1.03-5.72)*	5.40 (1.42-20.45)**
Relative weight gain 2-4 years (z-score)	0.53 (0.27-1.03)	0.51 (0.21-1.25)
Mother height (cm)	0.94 (0.75-1.17)	0.94 (0.75-1.17)
Pseudo R^2	0.14	0.24
Early Onset Overweight to Normal Weight		
Birth Weight (kg)	2.00 (0.84-4.72)	2.70 (0.93-7.88)
Relative weight gain 0-2 years (z-score)	2.39 (1.52-3.74)***	2.49 (1.52-4.08)***
Relative weight gain 2-4 years (z-score)	1.97 (1.23-3.17)**	2.27 (1.31-3.95)**
Mother height (cm)	1.00 (0.93-1.08)	1.01 (0.93-1.10)
Pseudo R^2	0.04	0.06

BMI = body mass index; * P<0.05; ** P< 0.01; *** P< 0.001.

Unadjusted = birth weight + relative weight gain 0-2 years + relative weight gain 2-4 years + mother height.

Adjusted= unadjusted + maternal education + social economic status + mother age + gestation age.

4.6 Discussion

We have shown that conditional relative weight gain in infancy and early childhood was significantly associated with different BMI trajectories, more especially early-onset obesity/overweight trajectories. For instance, a one standard deviation increase in z-score for conditional relative weight gain independent of linear growth between 0-2 years in girls resulted in an increased risk of 103% of following an early-onset obesity to morbidity obesity trajectory compared to those with a normal weight. It should be noted that weight gain is a combination of linear growth and changes in soft tissue, and conditional relative weight gain represents the weight gain that is not due to linear growth (De Beer et al., 2015). So the results of the analyses on conditional weight gain represent the weight gain in excess of what is expected from linear growth. The results show that excessive weight gain at any point during early childhood (before five years) almost exclusively determined, that an individual is likely to be in early-onset overweight or obese trajectory. This is consistent with a Brazilian study which reported that the amount of weight gain before five years of age was one of the most important predictors of overweight later in life (Fernandes et al., 2013).

Conversely to what has been observed in different settings, early (in infancy) conditional relative weight gain is more strongly associated with trajectories leading to obesity in adulthood than late (in childhood) conditional relative weight gain. Using more detailed longitudinal data and using BMI trajectories our findings are in contrast to the results reported from the five cohorts from low- and middle income countries, including South Africa, that faster weight gain in infancy and early childhood did not pose higher risk of body composition outcomes in adulthood (Adair et al., 2013; Adair et al., 2009). The analysis in the five used cross-sectional outcomes in adulthood while our analysis utilized BMI trajectories, hence capturing the overall longitudinal effects. Unlike results from other studies, birth weight and maternal height did not influence the subsequent BMI trajectory membership of cohort participants (Magee et al., 2013). Our results suggest the need of taking a life-course approach to understand how early life weight affects later life health outcomes, as it provides a better understanding of trajectories and health risk outcomes.

We reported a higher prevalence of overweight and obesity in girls than in boys across childhood and in young adulthood. Women were three times more likely to have excess weight than men in early adulthood, this is consistent with reported results on sex differences in obesity prevalence or incidence being higher girls and more than in boys and men in South Africa (Lundeen et al., 2016; Ng et al., 2014). The absolute prevalence of excess weight at 18 years of age was 33.8% in girls and 9.6% in boys and it was 46.6% in young women and 15.5% in young men at 23 years of age. These results are consistent with other studies in South African children, with girls having higher prevalence of excess weight than boys (Feeley et al., 2013; Lundeen et al., 2016; Pienaar, 2015; Puoane et al., 2013; Rossouw et al., 2012), but higher than those reported in most sub-Saharan Africa (SSA) countries (Muthuri et al., 2014). The sex differences in prevalence of overweight or obesity are reported across other SSA countries too (Ejike et al., 2010; Kamau et al., 2011; Muthuri et al., 2014; Salman et al., 2010). The prevalence rate of being obese or overweight (excess weight) in girls at 18 years in the current study is comparable to the rates in American children aged between 6 to 19, where a third of the children are obese or overweight (Flegal et al., 2012; Ogden et al., 2012). This also suggests that girls in the current study are at higher risk of transitioning into an obese trajectory, as they get older compared to boys. Individuals in trajectories characterized by high BMI values were at higher risk of being excess weight in early adulthood compared to those in a normal weight trajectory.

Since these trajectories represent early-onset obesity or overweight, previous studies have suggested that genetic and early life factors might be the driving force of these trajectories (Haga et al., 2012; Li et al., 2007). We confirmed that conditional relative weight gain between birth to five years influenced trajectory membership. Most of the reported factors that influence BMI trajectory membership, in American, Brazilian and European children, have been related to maternal factors such as maternal BMI, maternal gestational weight gain and maternal smoking during pregnancy and family social economic status (Börnhorst et al., 2016; Carling et al., 2015; Demment et al., 2014; Li et al., 2007; Lourenço et al., 2015).

Our results show that child's postnatal weight gain is also important in identifying those individuals in high-BMI trajectory groups and hence important factor to reduce future health risks. We speculate that conditional relative weight gain would have a similar important role in variation of BMI trajectory patterns in other populations, more especially in other black African populations.

The results also revealed that children belonging to early-onset obese or overweight were likely to have undesirable higher measure of overweight or obese in young adulthood. These results support the importance to consider the characteristics and risk factors associated with individual BMI trajectories, more especially at early stage of development. It also illustrates the strong tracking of weight gain which has been reported to be associated with multiple health risks such as childhood and adulthood obesity, blood pressure and metabolic syndrome (Börnhorst et al., 2016; Fernandes et al., 2013; Hejazi et al., 2009; Munthali et al., 2016; Perng et al., 2016).

Though BMI is not the perfect measure of body fat since it includes muscle mass and bone mass in defining fatness (Ortega et al., 2016), methods to get direct measures of body fat are expensive and difficult hence BMI, easy and cheap, is a suitable alternative for assessing general adiposity (Lear et al., 2007). Research has shown that BMI is strongly correlated with the most recommended methods of measuring body fat such as those from the dual-energy X-ray absorptiometry (DXA) (Anzolin et al., 2016; Gallagher et al., 1996; Goulding et al., 1996; Lindsay et al., 2001). Recently, body volume index (BVI), has been suggested to be the solution to the BMI shortfalls (Ajjimaporn et al., 2015; Tahrani et al., 2008). This is a ratio of total volume of the body to the volume of the abdomen; i.e. the ratio of total body fat to visceral fat.
Despite these innovations, these technologies remain expensive and BMI remains the most used measure of fatness more especially in low-and middle-income countries (Kromeyer-Hauschild et al., 2016).

This study has several strengths, the inclusion of detailed longitudinal data, which included early life factors, being first and foremost. This study is one of only a few that focuses on an African cohort, which provides a unique, in-depth understanding of how early life factors are linked to difference in BMI developmental patterns in South Africa. However, the early life factors investigated in this study explained only a small amount of variance in childhood BMI trajectories (< 25%) and it is important to consider other environmental and genetic aspects in future studies. We speculate the role of genetic ancestry, as well as changes in physical activities and dietary patterns in the past two decades may play a major role in determining trajectory membership.

Maternal factors that could have influenced the outcomes in this study, such as pre pregnancy weight or BMI, smoking and alcohol consumption before, during pregnancy, were not available in this study, and are a noted limitation. Other early life factors such as breastfeeding, dietary patterns data was also not available. Maternal weight on delivery was not collected, since it is influenced by other factors during pregnancy such as

gestational weight gain. It should be noted that at this stage it is not possible to determine whether the early life growth risk factors assessed in this study are causally involved in obesity development, which highlights the importance of further investigation to fully elucidate causality through techniques such as Mendelian randomization (Corbin et al., 2016). Due to the small sample size for boys in the early-onset overweight trajectory (1.3%), the association results for this particular trajectory membership could be affected, such as wider CIs resulting into tolerating false-positive estimates, and subsequently should be applied or inferred to other South African children with caution. These results can be generalized to other South African urban children setting but not for rural setting where the prevalence of obesity or overweight is relatively lower compared to the urban setting (Rossouw et al., 2012). Generalizability of our results to other African populations should be treated with caution since the sample for this study is from an urban South African population setting. Potential bias cannot be ruled out, because we had some lost to follow up at some years in this study

4.7 Conclusions

Regardless of the period of rapid weight gain, conditional relative weight gain increased the risk of an individual to belong to a BMI trajectory characterized with consistently elevated BMI. Consequently targeting appropriate weight gain in preschool years might lower the risk of the developing obesity and related metabolic disease risk in later life.

Supplementary Table 4-1: Descriptive statistics for BMI (mean (standard deviation)) development: The Birth to Twenty Plus Cohort

Age (Years)	Sample (Girls, Boys)	BMI (Kg/M^2), SD Girls (n=947)	BMI (Kg/M^2), SD Boys (n=877)	BMI (Kg/M^2), SD Study sample (n=1824)
5	670, 597	15.7 (1.56)	15.8 (1.36)	15.8 (1.47)
7	132, 146	16.0 (1.87)	15.9 (1.35)	15.9 (1.62)
8	396, 363	16.0 (2.16)	15.9 (1.53)	16.0 (1.88)
9	253, 249	16.7 (2.83)	16.4 (2.0)	16.6 (2.45)
10	158, 182	17.7 (3.35)	17.2 (2.63)	17.3 (2.99)
11	500, 468	18.1 (3.81)	17.4 (3.23)	17.8 (3.56)
12	673, 598	19.5 (4.05)	18.1 (3.08)	18.8 (3.69)
13	685, 609	20.6 (4.36)	18.6 (3.25)	19.6 (4.00)
14	746, 697	21.45 (4.45)	19.1 (3.37)	20.3 (4.14)
15	836, 756	22.2 (4.52)	19.6 (3.14)	21.0 (4.15)
16	717, 681	22.8 (4.59)	20.0 (3.03)	21.5 (4.16)
17	557, 501	23.3 (4.40)	20.4 (3.22)	21.9 (4.15)
18	426, 446	23.3 (4.65)	20.4 (2.77)	21.8 (4.08)

Supplementary Table 4-2: Odds ratios and 95% confidence intervals of the association of birth weight, BMI trajectories with excess weight status.

Trajectories	Unadjusted (OR)	Adjusted (OR)
Girls		
Obese or overweight BMI Trajectory		
Normal Weight	-	-
Early Onset Obese to Morbidity Obese	47.68 (6.44-353.22)***	44.83 (6.00 -334.86)***
Early Onset Obese to Overweight	7.24 (2.67-19.63)***	13.09 (2.92 -58.52)***
Late Onset Overweight	4.21 (2.41-7.33)***	4.73 (2.61-8.60)***
Birth Weight (kg)	1.66 (1.15-2.41)***	1.57 (0.99-2.48)
Pseudo R^2	0.11	0.12
Boys		
Obese or overweight BMI Trajectory		
Normal Weight	-	-
Early Onset Overweight to Obese	16.33 (3.10-86.07)**	13.62 (2.41-76.94)**
Early Onset Overweight to Normal Weight	7.93 (3.44-18.30)***	6.09 (2.33-15.93)***
Birth Weight (kg)	1.93 (1.19-3.12)	1.68 (0.93-3.02)
Pseudo R^2	0.08	0.07

** P< 0.01; *** P< 0.001

Adjusted for birth weight, maternal age, gestation age, social economic status and maternal education

OR = odds ratios

BMI = body mass index

4.8 References

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Chapter 5

Genetic risk score for adult body mass index associations with childhood and adolescent weight gain in an African population

in review: ***Genes & Nutrition***

Chapter 5: Genetic risk score for adult body mass index associations with childhood and adolescent weight gain in an African population

5.1 Preface – Empirical Paper 3

This article was submitted to *Genes & Nutrition*, where it is still under review. It was submitted as a third empirical research paper as follows: Richard J Munthali1, Venesa Pillay, Juliana Kagura, Liesl M Hendry, Shane A Norris, Ken K Ong, Felix R Day, Zané Lombard. Genetic risk score for adult body mass index associations with childhood and adolescent weight gain in an African population
(in review; ***Genes & Nutrition***).

This manuscript presents the last part of the study exploring the basis of obesity by investigating genetic risk of BMI as an indicator of obesity, which is one of the major risk factors of cardiovascular disease. It examines if a relationship exists between genetic risk score for adult BMI associated single nucleotide polymorphisms (SNPs) and longitudinal measurements of BMI. Genetic risk scores (GRSs) allow an overall assessment of genetic risk in complex traits. We investigated the relationship among participants' genetic risk score, conditional relative weight gain in infancy, early childhood, mid childhood and adolescence, and BMI at 18 years of age. This follows on the first and the second manuscripts, which reported distinct BMI trajectories and early life conditional relative weight gain being associated with early onset obese or overweight BMI trajectory membership. This manuscript elucidates the role of the combined genetic effect, in the form of a genetic risk score, on BMI trajectories membership. It is also important to explore whether the adult BMI associated SNPs in Caucasian population collectively have a relevant effect on BMI or obesity throughout the life-course in an African population, from birth to 18 years old of age. This genetic risk assessment could add value in understanding the variation of BMI development patterns across a range of ages hence help public health practitioners and policy makers to come up with obesity intervention strategies that target an appropriate growth period. We demonstrate this approach in context of BMI using a weighted genetic risk score constructed from the most robustly associated signals reported to date for BMI. We demonstrated how to construct and interpret a genetic risk score and how we can take advantage of it in studies that follow on after genome-wide analyses.

We selected genotyped and proxy SNPs (markers) from the most recent and largest meta-analysis of GWAS for adult BMI conducted by a number of consortia to construct a weighted genetic risk score in black South African subjects from the Birth to Twenty Plus Cohort.

The individual contributions by the candidate and any co-authors to the work are as follow:

RJM: Conception and design of the study, analyzed and interpreted data, wrote a first draft of the MS and approved final version to be published.

VP: Acquisition of genetic data, performing experiments, quality control assessment of data, critically assessed MS for intellectual content and approved final version to be published.

JK: Assisted with quality control assessment of data, critically assessed MS for intellectual content and approved final version to be published.

LMH: Helped with quality control assessment of genotype data, critically assessed MS for intellectual content and approved final version to be published.

SAN: Conception and design of the study critically assessed MS for intellectual content and approved final version to be published.

KKO: Assisted with study analysis, critically assessed data analysis, critically assessed MS for intellectual content and approved final version to be published.

FRD: Assisted with study analysis, critically assessed data analysis, critically assessed MS for intellectual content and approved final version to be published

ZL Conception and design of the study critically assessed MS for intellectual content and approved final version to be published.

5.2 Abstract

Ninety-seven independent single nucleotide polymorphisms (SNPs) are robustly associated with adult body mass index (BMI: kg/m^2) in Caucasian populations. The relevance of such variants in African populations at different stages of the life-course (such as childhood) is unclear. We tested whether a genetic risk score composed of the aforementioned SNPs was associated with BMI from infancy to early adulthood. We further tested whether this genetic effect was mediated by conditional relative weight gain at different growth periods. We used data from the Birth to Twenty Plus Cohort (Bt20+), for 971 urban South African black children from birth to18 years. DNA was genotyped using the Metabochip (Illumina) array. The weighted genetic risk score (wGRS) for BMI was constructed based on 71 of the 97 previously reported SNPs. The cross-sectional association between the wGRS and BMI strengthened with age from 5 to 18 years. The association was significant from 11 to 18 years, and peak effect sizes were observed at 13 and 14 years of age. Results from the interaction models showed a significant association between the wGRS and age, but not with sex. A higher wGRS was associated with the membership of a previously reported obese childhood BMI trajectory (relative risk =1.88; 95%CI: 1.28 to 2.76). Adolescent conditional relative weight gain mediated 56% of the association between wGRS and obesity risk at 18 years. The results suggest that genetic susceptibility to higher adult BMI is mediated through pathways involved in weight gain before and during adolescence. This supports the notion that prevention of adult obesity should begin early in life, especially avoiding excess weight gain before and during adolescence.

5.3 Introduction

Obesity, defined as having a (BMI) ≥ 30kg/m^2 in adults, is a growing public health concern globally, including in Africa (Kelly et al., 2008; Ng et al., 2014). Obesity is a complex multifactorial condition with both environmental and genetic factors playing a role. The prevalence of obesity varies widely across Africa ranging from 5.3% in Uganda to 30 % in Nigeria and 45.7% in South Africa (Ng et al., 2014). Genetic factors contribute about 40-70% of inter-individual variability in BMI (Visscher et al., 2012; Zaitlen et al., 2013), in addition to differences in environment and nutritional transitional stages playing a role in variability. Results from the largest and most recent meta-analysis of genome wide association studies (GWAS) identified 97 independent loci that influence adult BMI. The meta-analysis involved approximately 339, 224 individuals of predominantly European ancestry from 125 studies (Locke et al., 2015). Most of studies of the genetics of BMI focus on effects in adults and the role of genetics at other stages of the life-course, especially childhood, are not well studied. However, 15 genetic loci have been reported for BMI in childhood and most of these also influence adult BMI (Locke et al., 2015; Mei et al., 2012; Stergiakouli et al., 2014; Warrington et al., 2015).

Genetic associations seen in European populations are inconsistently replicated in African and Asian populations (Adeyemo et al., 2010; Fulford et al., 2015; Wen et al., 2012; Yako et al., 2015). Some studies have been conducted in African-American populations, but very few have been performed on the diverse populations within Africa, and even fewer studies using longitudinal data have been conducted (Adeyemo et al., 2010; Bollepalli et al., 2010; Grant et al., 2008; Ng et al., 2012). Exploring genetic variations in non-European ancestry populations may provide insights not only into establishing the exact risk variants but also into novel risk candidates. Repeat measurements and longitudinal data may enhance our understanding of the timing of such genetic influences, and in particular, life course studies of weight gain and adiposity may identify age-specific determinants (Hardy et al., 2010).

In a recent systematic review of genetic studies for BMI in Africans, Yako and colleagues showed that more than 300 SNPs in 42 genes have been investigated but very few positive findings were replicable. Although 36 of these 300 variants were validated through GWAS in non-African populations, only a handful of SNPs in or near *FTO* and *MC4R* have been verified to play a role in BMI variability in black South Africans, Ghanaians and Nigerians (Yako et al., 2015). The lack of replication may be due, in part, to the variation in genetic architecture between different ancestry populations. A recent study in Samoans reported a variant in the *CREBRF* gene (rs373863828) associated with an increased BMI of 1.36 kg/m^2 per risk allele, which explained 1.93% of the BMI variance in Samoans. Comparatively, the most robustly associated variant in Europeans (rs1558902 in *FTO* gene) has an effect size of 0.39kg/m^2 per risk allele and explains only 0.34% of the variance of BMI in Europeans. The *CREBRF* variant is common in Samoans but very rare in other populations, and were shown to be under positive selection in this population – emphasizing the need for studies in diverse populations (Minster et al., 2016).

Since most reported GWAS loci individually have small effect sizes, many follow-up (post-GWAS) studies have explored the role of combined risk allele scores (Belsky et al., 2013). For example, a study on a rural Gambian population reported that a combined allele score of 28 SNPs was positively associated with BMI and adult weight, whereas no association was observed when testing these SNPs in isolation (Fulford et al., 2015; Hennig et al., 2009). Such combined allele scores allude to the possibility of SNP-SNP interaction influencing disease outcome in such complex diseases. Few studies (both in African and non-African cohorts) have described the longitudinal associations between such combined allele scores on changes in BMI across childhood through to young adulthood (Fulford et al., 2015; Hung et al., 2015; Monnereau et al., 2016; Smith et al., 2015; Zhu et al., 2014). Furthermore, there is paucity of research to explore whether pathways related to weight gain at specific stages of early life mediate the associations between SNPs and adult BMI. The aims of this study were to determine the combined influence of known genetic risk loci (combined into a weighted genetic risk score (wGRS)) on BMI across infancy and late adolescence in a black South African cohort. We furthermore explored the possible mediating role of childhood and adolescent weight gain on this genetic effect, and also examined the association between the wGRS with previously identified childhood BMI trajectories in this cohort (Munthali et al., 2016).

5.4 Subjects and Methods

5.4.1 Study sample

The Birth to Twenty Plus Cohort (Bt20+) is a prospective cohort study among black urban South African children born in 1990. The cohort is focused on the health and development of children born in an urban township of Soweto in Johannesburg, South Africa. Since recruitment, the participants have been contacted regularly and longitudinal data and DNA samples were collected at several time points. Details on the cohort have been described elsewhere (Richter et al., 2007). Approval for the Bt20+ study was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg (Certificate number M0101556). In addition this committee approved the use of genetic data for this study under certificate number M1411115. Participants or their parents/caregivers where participants were minor gave informed consent at the beginning of each data collection session throughout the study.

5.4.2 Procedures

Genotyping

DNA was extracted using the standard salting-out method (Miller et al., 1988) from blood samples at age of 13 years of age and genotyped using the Metabochip (Illumina, San Diego, California, USA) at the DNA Technologies Core of the University of California Davis (California, USA) (Voight et al., 2012).

Data were generated on 196,725 SNPs in 1248 participants before quality control (QC) using PLINK version 1.9 (Purcell et al., 2007). 68,961 SNPs were excluded due to a missing rate >2%, minor allele frequency <1%, or departure from Hardy-Weinberg equilibrium ($P<1\times10^{-5}$) Subsequently, 272 samples were excluded due to genotype missing rate >3%, extreme heterozygosity between genotypes (± 3 standard deviations (SD) from the mean), discordant sex assignment, identity by descent (IBD) score > 0.1875, duplicate samples, or a population outlier based on principal component analysis. Five individuals did not have data on childhood BMI trajectory and were also excluded. The final dataset comprised of 127,764 SNPs in 971 participants.

Genetic risk score construction

Of the 97 independent adult BMI associated SNPs reported by Locke et al. (Locke et al., 2015), 69 were present in our dataset and we identified proxies ($r^2>0.8$) for a further two SNPs, resulting in 71 SNPs for analysis (Supplemental Table 1 & Figure 5-1).

Genotypes at these 71 variant positions were combined to construct a weighted BMI genetic risk score (wGRS). Weighting was performed by multiplying the number of BMI-increasing alleles at each locus (0, 1, or 2) in each individual by the corresponding effect size (in kg/m^2 per allele) as reported by Locke et al. (Locke et al., 2015). In the absence of GWAS meta-analysis data in African populations, we used the BMI effect estimates reported in European ancestry populations. The wGRS was rescaled to standard deviation scores (SDS), Z-scores (wGRSz), by calculating (individual wGRS value *minus* population mean wGRS)/population standard deviation wGRS. The Z-score transformed wGRS were utilized to account for the variation in number of risk alleles constituting the wGRS (Malik et al., 2014; Monnereau et al., 2016).

Assessment of growth

Trained research assistants measured birth weight, postnatal weight and height. Weight was measured using a digital scale to the nearest 0.1 kg with participants in light clothes and without shoes. A wall-mounted stadiometer (Holtain, UK) was used to measure standing height to the nearest 0.1 cm. Weight and height values were collected annually from birth up to 23 years and were used to calculate BMI as weight (kg) divided by height (m) squared.

BMI Z-scores were computed using the World Health Organization (WHO) growth standards. (De Onis, 2006) Conditional weight gain independent of height gain ("conditional relative weight gain") was calculated for each growth period as standardized residuals (SR) from sex-specific linear regressions of current weight on prior weights, heights and current height in infancy (0-2 years), early-childhood (2-5 years), mid-childhood (5-8 years) and adolescence (8-15 years) (Adair et al., 2013; Keijzer-Veen et al., 2005).

Childhood adiposity trajectories

We previously identified three and four distinct adiposity trajectories in boys and girls respectively, in the same group of Bt20+ participants, using latent class growth mixture modeling (LCGMM) (Munthali et al., 2016). Adiposity trajectory membership was assigned depending on the high probability of belonging to a particular trajectory depending on Bayesian posterior probabilities. Trajectory membership was graded by

increasing risk of childhood obesity and used as an outcome variable in multinomial logistic regression models with wGRSz as an independent variable. Upon merging data for girls and boys, trajectories with similar patterns were combined, with one trajectory (early onset obese to overweight) comprising of girls only.

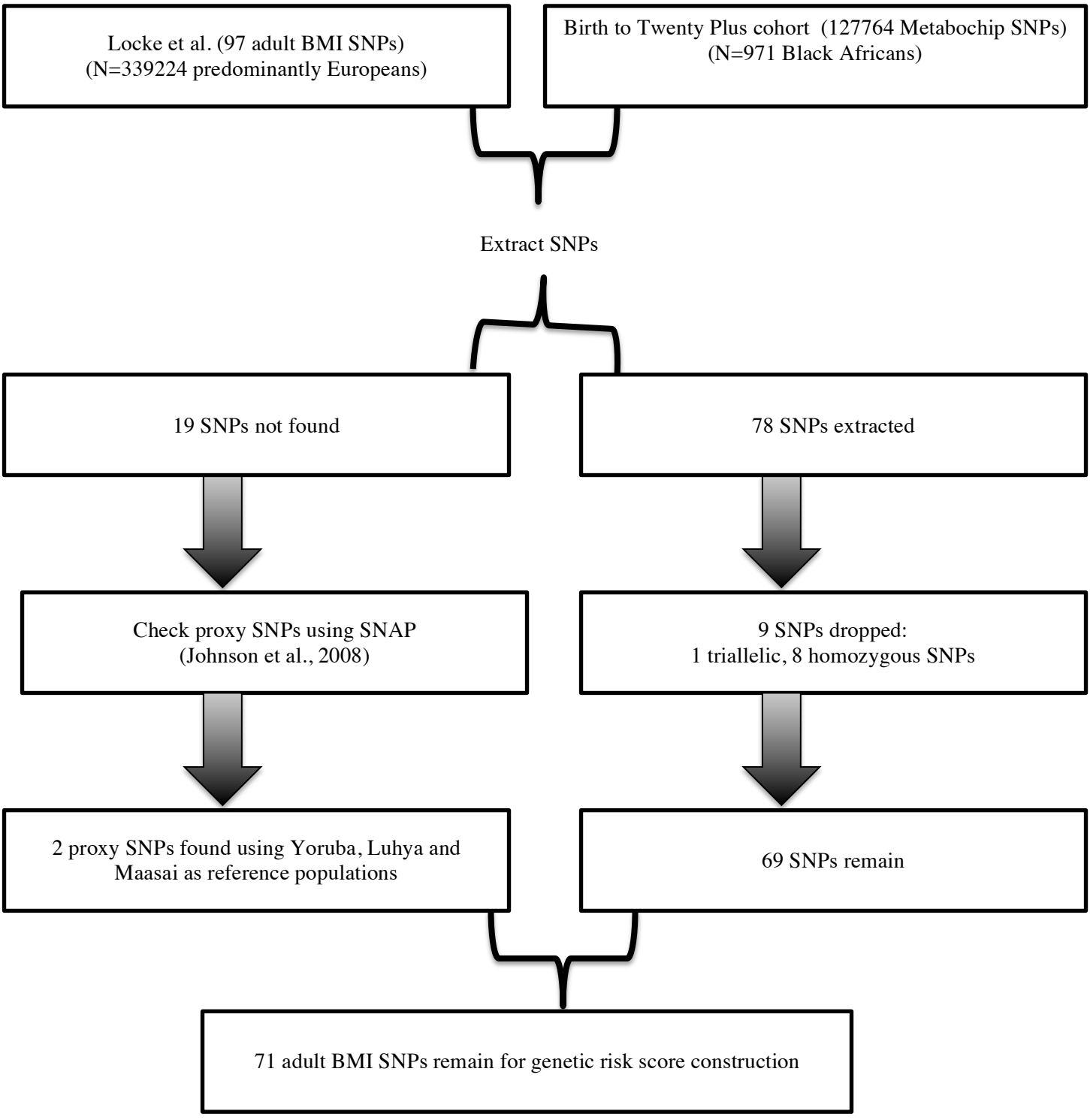


Figure 5-1: Flowchart displaying the selection of BMI related SNPs for inclusion in the weighted BMI genetic risk score

5.4.3 Statistical Analysis

Linear, mixed and multinomial logistic regression models were used to examine the cross-sectional and longitudinal associations between wGRSz and BMI from infancy to early adulthood, adjusting for sex. It was previously reported that the patterns of missing data on weight and height are likely to be missing at random (Munthali et al., 2016); we still reduced the potential bias by not excluding individuals who did not have data at every time point.

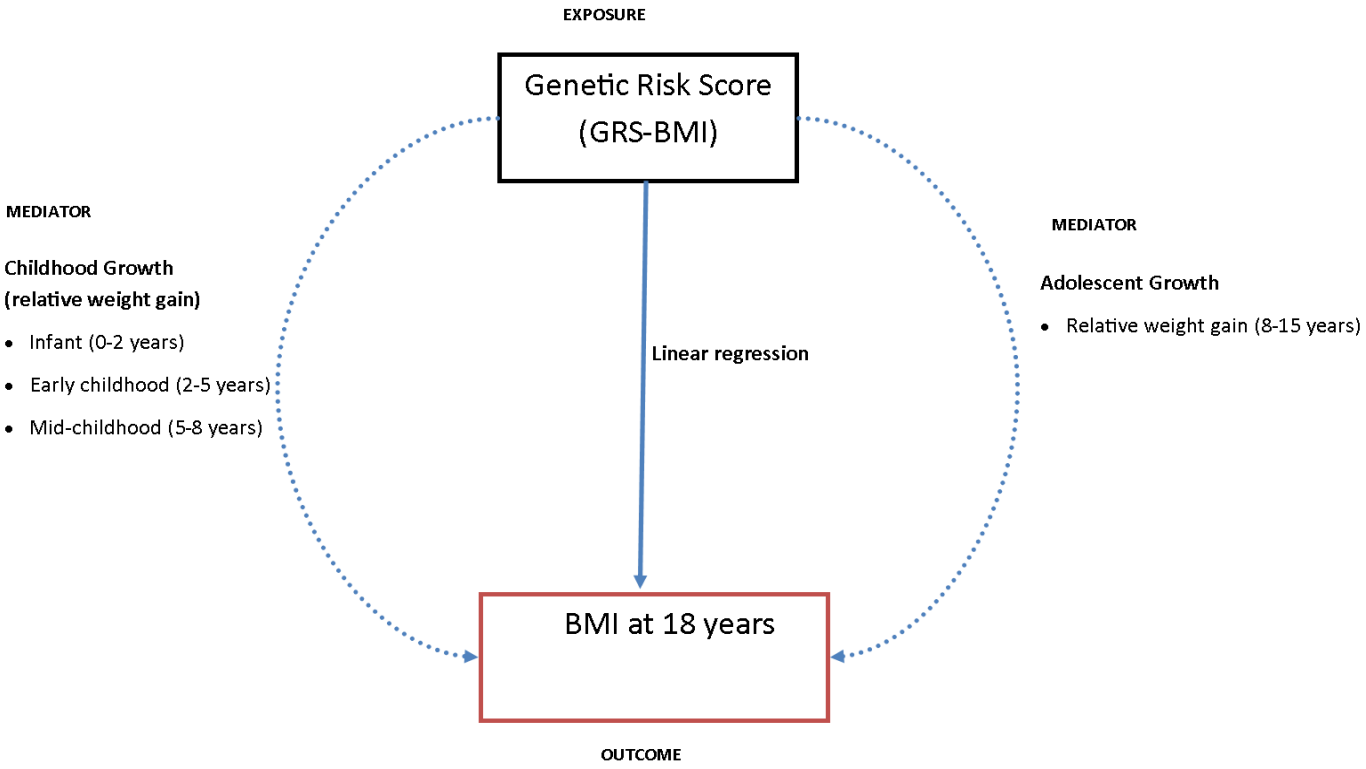


Figure 5-2: Schematic diagram for the mediation analysis, the role of childhood and adolescent growth on association between wGRS and BMI at 18 years of age.

The wGRSz*age, wGRSz*age^2 and wGRSz*age^3, these interaction terms were calculated to capture both linear and non-linear growth interactions with age; wGRSz*age interactions can be interpreted as effects of linear change in BMI Z-score with age. To investigate mediation, a Sobel test (Sobel, 1982) was used to examine the role of infancy, childhood and adolescent growth (conditional relative weight gain) on the association between wGRSz and BMI Z-scores in early adulthood (Figure 5-2). We explored the associations between wGRSz and BMI trajectory membership using multinomial logistic regressions. All analyses were performed using STATA version 13.0 (STATA Corp, TEXAS). A P-value < 0.05 was considered statistically significant.

5.5 Results

BMI at 18 years was lower in young men than young women (20.3 kg/m^2 vs. 23.3 kg/m^2, Table 5-1). The frequencies of wGRS followed an approximately normal distribution (Supplemental Figure 5-1). Cross-sectional associations between wGRS and BMI increased with age at measurement up to 14 years then declined, Figure 5-3, and were statistically significant between ages 11 to 18 years, with peak effect sizes at 13 and 14 years old. For each SD higher wGRS, BMI was higher by 0.15 SDS at 13 and 14 years old. The wGRS was not associated with birth weight.

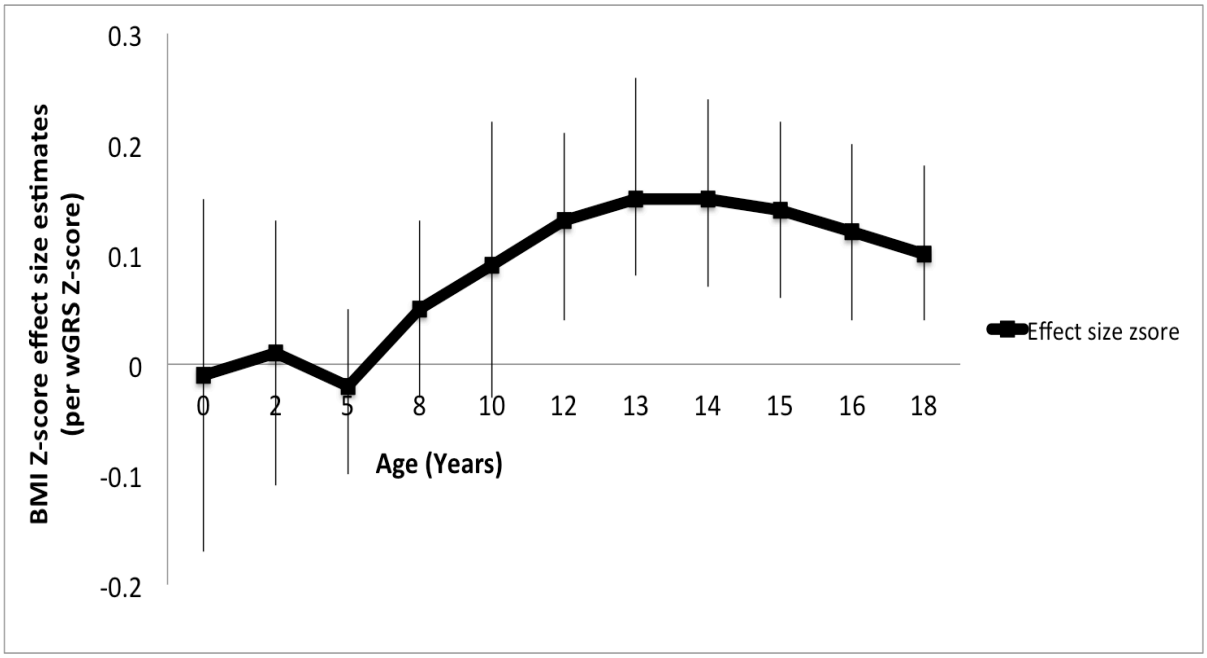


Figure 5-3: Association between the weighted genetic risk Z-score and BMI Z-score at each age (error bars indicate 95% confidence intervals)

Significant interactions with wGRS on BMI SDS were observed for 'Age' (β=0.01 SDS per SD per year; 95% CI: 0.006 to 0.012), 'Age^2' (p<0.001) and 'Age^3' (p<0.001) terms but there was no such interaction with 'Sex'. The wGRS positively predicted membership of the previously reported 'obese BMI trajectory' (relative risk ratio =1.88; 95%CI: 1.28 to 2.76).

Mediation by childhood and adolescent weight gain

The Sobel tests showed significant mediating effects of conditional relative weight gain during adolescence (56% of total effect) on the association between the wGRSz and BMI at 18 years (Figure 5-4). Although not significant, it is interesting to note that mid-childhood conditional relative weight gain mediated a further 28% of this association (P=0.15). Conditional relative weight gain in infancy (0.0% of total effect mediated) and early childhood (1.7% of total effect mediated) did not appear to mediate the wGRSz association with adult BMI (Table 5-4).

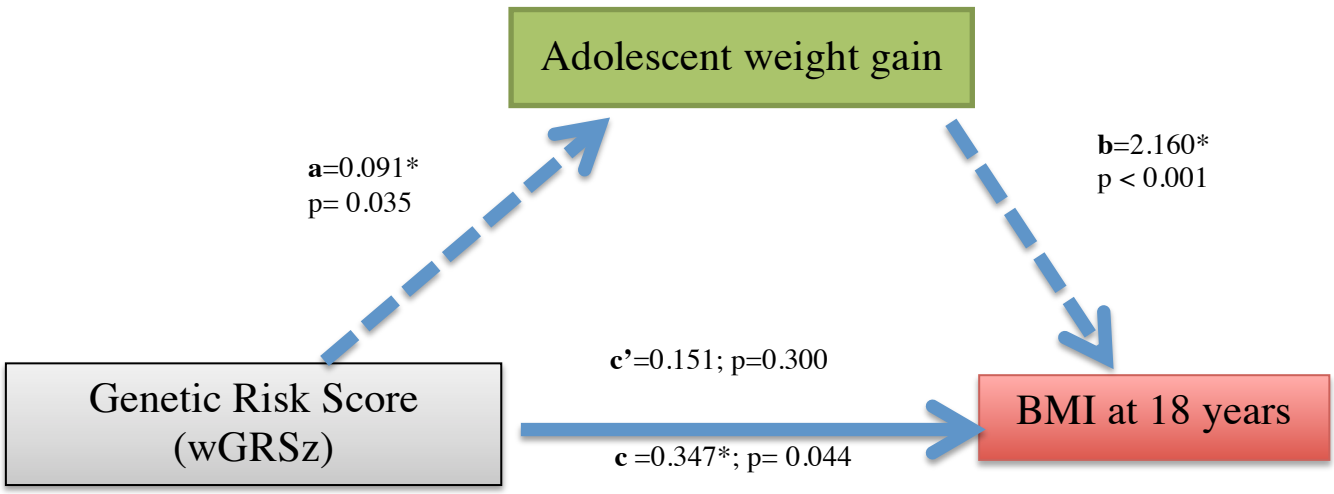


Figure 5-4: Impact of weighted genetic risk score (GRS) on BMI at 18 years via adolescent conditional relative weight gain.

Notes: Path **a** is the direct effect of the wGRS on mediator (Adolescent weight gain). Path **b** is the mediator's effect on the outcome (BMI at 18 years) eliminating the effect of wGRS. The total effect of wGRS on BMI at 18 years is **c=c' +ab**. Where **ab** is the indirect effect of wGRS on BMI at 18 years via adolescent weight gain.

Table 5-1: Comparing study characteristics between boys and girls study participants.

	Age (years)											
	0		**2**		**5**		**8**		**15**		**18**	
Variable	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Height (cm)	67.73	66.52	83.7*	82.9	107.6	107.2	125.1	124.3	165.5*	158.7	170.8*	159.7
Weight (kg)	3.1*	3	11.4*	11.2	18.2	18	25.1	25.1	53.7*	56.3	59.4	59.5
BMI (kg/m^2)	17.7	17.4	16.4	16.3	15.8	15.6	16	16.1	19.53*	22.33	20.3*	23.3
BMI Z-score	0.4*	0.5	0.2	0.4	0.3*	0.1	0	0	-0.5*	0.32	-0.7*	0.4
Sample size (n)	516	453	346	305	413	356	304	257	476	436	493	429

M: Male; F: Female and *P<0.05

Table 5-2: Path coefficients, direct and total effect estimates: Mediation by conditional relative weight gain on the association between the weighted genetic risk score (wGRS) and BMI at 18 years of age

Weight gain period	**Sample Size (n)**	**[a]wGRS to Weight Gain**	**[b]Weight Gain to BMI at 18 years**	**[c]wGRS to BMI at 18 years (total)**	**wGRS to BMI at 18 years (direct)**	**[d]Proportion mediated**	**Sobel P-value**
Infancy (0-2 years)	630	0.007	1.147*	0.460*	0.452*	0.017	0.858
Early childhood (2-5 years)	574	-0.027	0.884*	0.435*	0.459*	-0.055	0.530
Mid-childhood (5-8 years)	448	0.067	1.351*	0.329*	0.238	0.28	0.151
Adolescence (8-15 years)	**514**	**0.091***	**2.160***	**0.347***	**0.151**	**0.56**	**0.037**

*P<0.05

Notes: [a]wGRS to Weight Gain is measured as standardized residuals (SR) of weight gain per SD of wGRS

[b]Weight Gain to BMI is measured as BMI SDS per SR of weight gain

[c]wGRS to BMI at 18 is measured as BMI SDS per SD of wGRS

[d]Proportion of the total wGRS to BMI at 18 association that is mediated by conditional relative weight gain

5.6 Discussion

In an African population, followed longitudinal from birth to 18 years, we explored the cumulative effect of adult BMI-associated risk variants identified in Caucasian samples on the trajectory of childhood BMI. Our results show that the genetic variants that influence adult BMI become increasingly important during the adolescent years (11 to 18 years, peaking between 13 and 14 years). The effects of these BMI-associated variants on weight gain during this period appear to mediate the genetic effects on adult BMI. This suggests that the aetiology of obesity follows an increasingly adult pattern starting at age 11 (and possibly earlier in other cohorts. The wGRSz was not associated with BMI increase in infancy and early childhood, indicating that this risk assessment tool is perhaps not appropriate for use during this period of development. It might also reflect that rapid weight gain in infancy and early childhood are unlikely pathways through which

genetic risk for obesity manifests. The observation that there was no significant interaction between the genetic score and sex suggests that the genetic aetiology of obesity is not substantially different between the sexes, despite the observed differences in BMI trajectories (Munthali et al., 2016). In South Africa obesity prevalence is much higher in women (70%) than men (40%) across the life-course, and further investigation in this sex-specific observation is needed (Lundeen, Norris, Adair, et al., 2016; Ng et al., 2014).

Only one previous study utilized wGRS on longitudinal BMI in childhood in Africans, which reported that a genetic risk score was associated with adult weight but not with birth weight, similar to what was observed in this current study (Fulford et al., 2015). It was also reported that the effect of a wGRS on BMI increased with age in this Gambian population, which is also in line with our current findings.

Similar study designs have been reported in European populations. Among 3,975 Dutch children with a median age of 6 years, the peak effect of the genetic score on BMI was observed at age 6, (Monnereau et al., 2016) which contrasts with the non-significant inverse trend with BMI at age 5 in our current study. In 9,328 British and Australian children, a GRS of 32 adult BMI associated SNPs was positively associated with BMI changes from birth to 16 years (Warrington et al., 2013). Another study in 652 Norwegian children, followed from age 4 years through age 8 years, reported that a similar 32-SNP GRS was associated with accelerated childhood weight gain during this period, although appetite traits did not appear to mediate these associations (Steinsbekk et al., 2016). In 5,906 Native Americans of predominantly Pima Indian heritage aged between 5 to 45 years, 36 SNPs were associated with childhood BMI and allelic risk score, and a subset from these 36 SNPs was associated with increased birth weight and greater rates of BMI change in childhood (Hohenadel et al., 2016). These results might reflect differences in the obesogenic environments between children in different parts of the globe, especially as it is thought that large energy surpluses in early life could result in earlier puberty and adult development (Lundeen, Norris, Martorell, et al., 2016). We cannot rule out the possibility that the GRS may mediate earlier effects on weight gain and BMI changes in more obesogenic settings. There is consistent evidence that genetic susceptibility to higher adult BMI promotes faster weight gain in childhood in different populations, but specific influences (perhaps related to local environments) may determine the onset and peak timing of these effects.

Other factors might contribute to the apparent differences in the timing of effects of GRS on weight gain between Caucasian and African population, in particular the marked differences in genetic

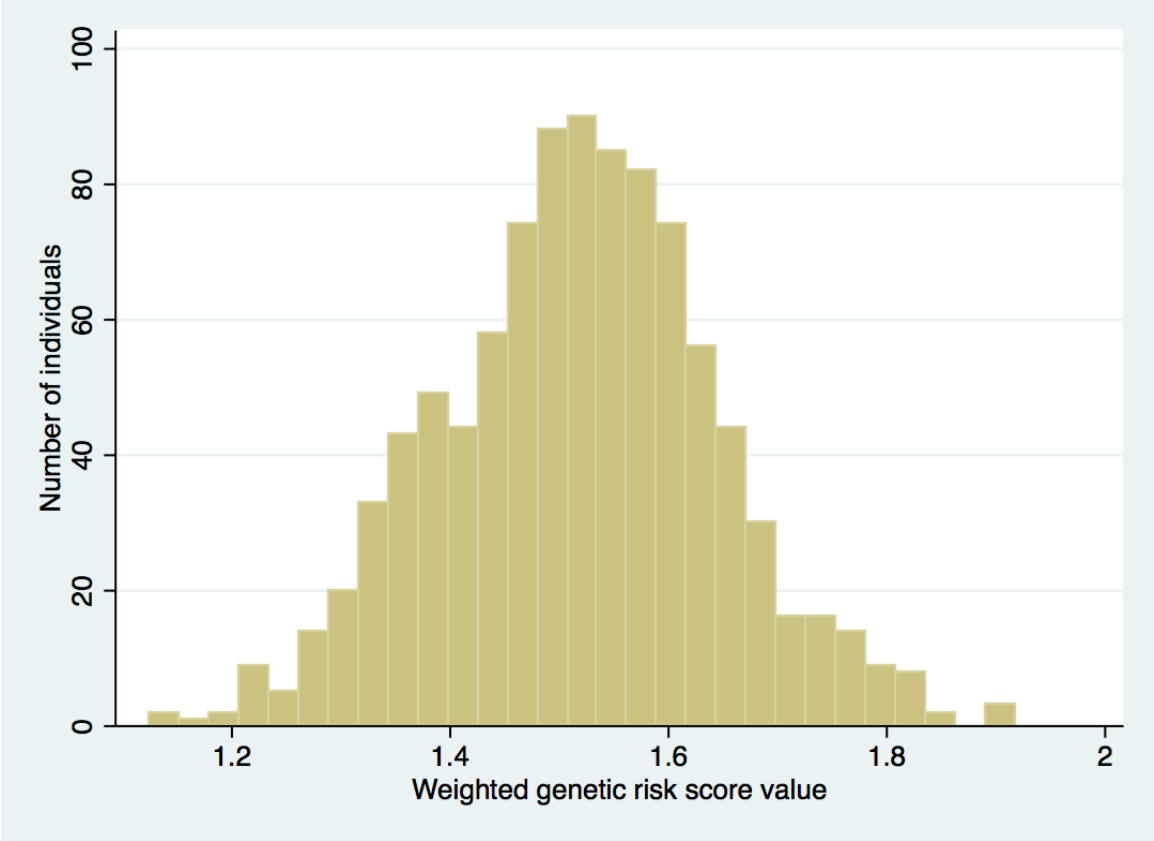
architecture among these populations (Teo et al., 2010). It has also been suggested that such differences could contribute to the inconsistent replication of BMI associated loci in black African populations (Adeyemo et al., 2010; Fulford et al., 2015; Hennig et al., 2009; Yako et al., 2015).

There has been an increasing interest in understanding factors influencing the variation in BMI growth patterns from childhood to adulthood. Our current findings have shown that a higher wGRSz puts an individual at increased risk of being a member of a growth trajectory characterized by high BMI values across childhood and adolescence. This is consistent with the results from the cross-sectional association between wGRS and BMI, but there is clearly a role for other factors - early life growth, socio-demographic, socioeconomic, ethnicity and gender (Magee et al., 2013; Rossi et al., 2011; Roy et al., 2015; Ziyab et al., 2014). There may also be genetic factors that are specific to this age range: a recent study in 1,229 Mexican American adolescents reported that genetic polymorphisms in genes related to risk-taking behaviors such as those in *COMT*, *HTR2A* and *SLC6A3A* were associated with BMI trajectories membership (Zhao et al., 2016). As our study considered variants previously associated with adult BMI, discovery of these types of effects was beyond the scope of the current study, but genome-wide studies of adiposity at these ages may reveal different SNPs and associated with adiposity biology. In particular, using defined growth trajectories as the outcome of interest has the specific benefit of reducing modeling complexity in longitudinal genetic data analysis. This method has a potential clinical significance, as it would be able to pinpoint the most at risk subgroups (based on their GRS), and could be utilized to predict disease progression (Musolf et al., 2014; Sakai et al., 2010).

The main strength of our study is that longitudinal data were merged with a wGRS, to increase the power of detecting significant associations. The use of different longitudinal methods to understand the influence of the genetic risk score on BMI suggested the likely utility of modelling BMI trajectories in future genetic studies in large sample sizes. This approach would also allow harmonization between studies that collected data at different time points.
The study has a few weaknesses. Firstly, only a single African population was studied here meaning that the generalizability of our findings to other African populations will need to be determined. Secondly, the BMI genetic risk score was generated from predominantly Caucasian studies; the GRS constructed may not be a true reflection of BMI genetic risk in African populations. There is a need for large scale GWAS data in African populations and do meta-analysis analyses of GWAS of obesity in Africa. This would help to come up with variants from an African perspective for constructing a GRS. Potential confounders such as maternal pre-pregnancy BMI and other maternal

factors were unavailable and were not considered in the current study. Finally, the genetic risk score approach gives us an insight into the overall genetic susceptibility but much larger studies will be required to examine effects of specific individual genes.

In conclusion, we observed a significant combined effect of adult BMI associated genetic variants on childhood and adolescent BMI and childhood adiposity trajectories in a black South African population. Therefore the adult BMI associated SNPs in Caucasian population collectively have relevance effect on BMI or obesity throughout the life-course in an African population. There seems to be some transferability of these "Caucasian" adult obesity risk SNPs to an African cohort, even in lieu of direct association testing. The observed mediation of the genetic susceptibility to adult obesity by rates of weight gain in mid to late childhood suggests that intervention strategies and treatments that target early in life growth periods might be effective to prevent adult obesity.



Supplemental Figure 5-1: Distribution of the weighted genetic risk score in the Birth to Twenty Plus Cohort

Supplementary Table 5-1 -Details of the 71 SNPs used to construct the weighted BMI genetic risk score

SNP	Chr	Position (bp/hg19)	Nearest gene	Effect Allele	Other Allele	A1 (minor_ btt20+)	Minor (all_1000g)	CEU allele freq	YRI allele freq	Locke effect allele freq
rs1000940	17	5,223,976	*RABEP1*	G	A	G	G	0.35	0.23	0.32
rs10408163(1)	19	52,260,843	*ZC3H4*	A	G	G	G	0.69	0.10	0.65
rs10733682	9	128,500,735	*LMX1B*	A	G	A	G	0.51	0.74	0.48
rs10968576	9	28,404,339	*LINGO2*	G	A	G	G	0.31	0.15	0.32
rs11126666	2	26,782,315	*KCNK3*	A	G	A	A	0.28	0.17	0.28
rs11583200	1	50,332,407	*ELAVL4*	C	T	T	T	0.63	0.28	0.41
rs1167827	7	75,001,105	*HIP1*	G	A	A	G	0.54	0.95	0.56
rs11688816	2	62,906,552	*EHBP1*	G	A	A	A	0.53	0.35	0.53
rs11847697	14	29,584,863	*PRKD1*	T	C	T	T	0.05	0.38	0.10
rs12286929	11	114,527,614	*CADM1*	G	A	A	G	0.49	0.59	0.52
rs12401738	1	78,219,349	*FUBP1*	A	G	A	A	0.32	0.09	0.35
rs12446632	16	19,842,890	*GPRC5B*	G	A	A	A	0.12	0.09	0.87
rs12566985	1	74,774,781	*FPGT-TNNI3K*	G	A	A	A	0.53	0.17	0.46
rs12940622	17	76,230,166	*RPTOR*	G	A	G	A	0.43	0.60	0.57
rs13021737	2	622,348	*TMEM18*	G	A	A	G	0.82	0.93	0.83
rs13191362	6	162,953,340	*PARK2*	A	G	G	G	0.12	0.03	0.88
rs1441264	13	78,478,920	*MIR548A2*	A	G	G	A	0.65	0.70	0.61
rs1460676	2	164,275,935	*FIGN*	C	T	C	C	0.19	0.25	0.18
rs1516725	3	187,306,698	*ETV5*	C	T	T	C	0.86	0.82	0.87
rs1528435	2	181,259,207	*UBE2E3*	T	C	C	T	0.6	0.63	0.63
rs1558902	16	52,361,075	*FTO*	A	T	A	A	0.44	0.05	0.41
rs16907751	8	81,538,012	*ZBTB10*	C	T	T	T	0.13	0.06	0.91
rs16951275	15	65,864,222	*MAP2K5*	T	C	C	C	0.23	0.42	0.77
rs17001561(2)	4	77,348,592	*SCARB2*	G	A	A	A	0.16	0.12	0.15
rs17024393	1	109,956,211	*GNAT2*	C	T	C	C	0.04	0.08	0.04
rs17405819	8	76,969,139	*HNF4G*	T	C	C	C	0.29	0.03	0.70
rs17724992	19	18,315,825	*PGPEP1*	A	G	G	G	0.27	0.08	0.74
rs1808579	18	19,358,886	*C18orf8*	C	T	T	T	0.46	0.45	0.53
rs1928295	9	119,418,304	*TLR4*	T	C	C	C	0.43	0.44	0.55
rs2033732	8	85,242,264	*RALYL*	C	T	T	C	0.76	0.9	0.75
rs205262	6	34,671,142	*C6orf106*	G	A	A	G	0.27	0.72	0.29
rs2075650	19	50,087,459	*TOMM40*	A	G	G	G	0.13	0.17	0.85
rs2080454	16	47,620,091	*CBLN1*	C	A	A	A	0.61	0.32	0.41
rs2112347	5	75,050,998	*POC5*	T	G	G	G	0.38	0.51	0.62
rs2121279	2	142,759,755	*LRP1B*	T	C	T	T	0.13	0.02	0.15

rs2176040	2	226,801,046	*LOC646736*	A	G	A	G	0.63	0.67	0.36
rs2176598	11	43,820,854	*HSD17B12*	T	C	T	C	0.75	0.62	0.26
rs2207139	6	50,953,449	*TFAP2B*	G	A	G	G	0.18	0.07	0.18
rs2245368	7	76,446,079	*PMS2L11*	C	T	C	T	0.79	0.82	0.19
rs2287019	19	50,894,012	*QPCTL*	C	T	T	T	0.19	0.12	0.81
rs2365389	3	61,211,502	*FHIT*	C	T	C	T	0.40	0.85	0.57
rs2820292	1	200,050,910	*NAV1*	C	A	C	C	0.55	0.38	0.55
rs2836754	21	39,213,610	*ETS2*	C	T	C	C	0.60	0.29	0.60
rs29941	19	39,001,372	*KCTD15*	G	A	A	G	0.68	0.86	0.67
rs3101336	1	72,523,773	*NEGR1*	C	T	C	C	0.63	0.53	0.61
rs3736485	15	49,535,902	*DMXL2*	A	G	G	G	0.54	0.39	0.46
rs3817334	11	47,607,569	*MTCH2*	T	C	T	T	0.42	0.23	0.40
rs3888190	16	28,796,987	*ATP2A1*	A	C	A	A	0.34	0.20	0.40
rs4256980	11	8,630,515	*TRIM66*	G	C	G	G	0.63	0.51	0.64
rs4740619	9	15,624,326	*C9orf93*	T	C	C	C	0.48	0.50	0.54
rs4787491	16	29,922,838	*INO80E*	G	A	G	G	0.53	0.53	0.51
rs543874	1	176,156,103	*SEC16B*	G	A	G	G	0.20	0.28	0.20
rs6465468	7	95,007,450	*ASB4*	T	G	T	T	0.30	0.11	0.30
rs6477694	9	110,972,163	*EPB41L4B*	C	T	T	T	0.65	0.59	0.37
rs6567160	18	55,980,115	*MC4R*	C	T	C	C	0.23	0.24	0.24
rs657452	1	49,362,434	*AGBL4*	A	G	G	G	0.63	0.44	0.40
rs6804842	3	25,081,441	*RARB*	G	A	G	G	0.57	0.37	0.57
rs7138803	12	48,533,735	*BCDIN3D*	A	G	A	A	0.34	0.18	0.38
rs7141420	14	78,969,207	*NRXN3*	T	C	C	T	0.52	0.58	0.53
rs7164727	15	70,881,044	*LOC100287559*	T	C	T	T	0.68	0.30	0.67
rs7239883	18	38,401,669	*LOC284260*	G	A	A	A	0.58	0.53	0.39
rs7243357	18	55,034,299	*GRP*	T	G	G	G	0.19	0.15	0.81
rs7599312	2	213,121,476	*ERBB4*	G	A	A	A	0.26	0.38	0.72
rs7715256	5	153,518,086	*GALNT10*	G	T	G	T	0.57	0.66	0.42
rs7899106	10	87,400,884	*GRID1*	G	A	G	G	0.04	0.16	0.06
rs7903146	10	114,748,339	*TCF7L2*	C	T	T	T	0.31	0.28	0.71
rs9374842	6	120,227,364	*LOC285762*	T	C	C	T	0.73	0.79	0.75
rs9400239	6	109,084,356	*FOXO3*	C	T	C	C	0.66	0.21	0.67
rs977747	1	47,457,264	*TAL1*	T	G	G	G	0.61	0.29	0.40
rs9914578	17	1,951,886	*SMG6*	G	C	C	G	0.21	0.60	0.23
rs9925964	16	31,037,396	*KAT8*	A	G	G	G	0.40	0.09	0.62

Notes

SNP: Single nucleotide polymorphism

Chr: Chromosome

Effect Allele: The effect allele as reported by Locke et al. (2015)

Other Allele: Other allele as reported by Locke et al. (2015)

A1 (minor_btt20+): Minor allele in the current study, from the Bt20+ dataset

Minor (all_1000g): Minor allele as reported in the 1000 Genomes dataset

CEU allele freq: Reference European allele frequency from 1000 Genomes (obtained from Locke et al. 2015)

YRI allele freq: African allele (Yoruba) frequency of the effect allele (as per Locke et al. 2015) from 1000 Genomes dataset

Locke effect allele freq: Allele frequency of the effect allele in Locke et al. (2015) dataset

rs10408163 (1) proxy SNP for rs3810291 from Locke et al. (2015) dataset

rs17001561 (2) proxy SNP for rs17001654 from Locke et al. (2015) dataset

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Part 3

Discussion and Implications

Chapter 6: Discussion

Preface to the chapter

The reader may refer to specific Chapters for preliminary discussions on the key findings of this thesis. Here, I would like to focus on the summary of the findings, emerging themes and how they form one story, public health implications and methodological considerations of these findings on this study.

6.1 Summary of study findings

The main aim of this study was to use longitudinal data analysis to explore the influence of genetic variants on obesity and explore early life growth factors and future health risks of BMI growth patterns from 5 to 18 years old. The consolidated findings in line with the empirical chapters are outlined in table 6-1.

Table 6-1: Consolidated study findings

Chapter	Objective	Key findings
3	**1a &1b; Identify distinct adiposity trajectories**	Using the latent class growth mixture modelling (LCGMM), I identified three (normal weight, early onset overweight to normal and early onset overweight to obese) and four (normal weight, late onset overweight, early onset obesity to overweight and early onset obesity to morbidly obese) sex-specific distinct adiposity trajectories in boys and girls, respectively.
3	**1c; Find the prevalence of elevated blood pressure at 18 years old**	The overall prevalence of elevated blood pressure in late adolescence was 34.91 % (39.43 % boys and 30.38 % girls). This was relatively higher than that reported in children from other African countries such as in Brazzaville Congo, Ghana and Egypt.

3	**1d; Explore associations between the distinct adiposity trajectories and elevated BP in late adolescence**	Boys and girls in the early onset obesity or overweight adiposity trajectories membership had higher BP values in late adolescence. Girls in early onset obesity trajectories had an increased risk of elevated BP with odds ratio of 2.18 (95 % confidence interval 1.31 to 4.20; P<0.05) and 1.95 (1.01 to 3.77; P<0.05) compared to their counterparts in a normal weight trajectory in late adolescence
4	**2a; Assess the association between early-life growth factors, such as relative conditional weight gain and birth weight, and adiposity trajectories from ages 5 to 18 years**	Faster conditional relative weight in infancy predicted early onset obesity to morbid obesity trajectory membership in girls and early onset overweight to obesity membership in boys compared to those in normal weight trajectory. Birth weight and maternal height were not predictors of any of the adiposity trajectories membership in both girls and boys.
4	**2b & 2c; Explore association between adiposity trajectory membership and risk of overweight or obesity in young adulthood at the age of 23 years**	The prevalence of overweight or obesity in early adulthood at the age of 23 years was relatively higher in girls 46.6% in girls and 15.5% in boys. Girls and boys in early onset obesity or overweight trajectories had an increased risk of obesity in young adulthood compared to their peers in normal weight trajectory.
5	**3a & 3b; Examine genetic influence on BMI changes from birth to 18 years**	The cross sectional association between genetic risk score and body mass index strengthened with age from 5 to 18 years, with peak associations observed at 13 and 14 years. There were significant interactions between age and genetic risk score but not significant between sex and

		genetic risk score. The genetic risk score was not associated with birth weight.
5	**3c; Determine whether the genetic effect on BMI in young adulthood was mediated childhood and adolescent growth**	Conditional relative weight gain during adolescence and mid-childhood mediated the genetic effects on adult BMI but conditional weight gain in early childhood and infancy did not.
5	**3d; Examine if the genetic risk score for adult BMI predicted childhood adiposity trajectories membership**	A higher genetic risk score had an 88% increase risk of driving an individual to follow 'an obese BMI trajectory' membership compared to following a normal weight trajectory.

6.2 Null Hypotheses re-visited

From the findings of the study as presented in the empirical chapters, I revisited the hypotheses for this thesis.

6.3.1 Hypothesis 1.3.2.i: There are no different sex-specific distinct adiposity trajectories between 5 and 18 years of age.

I rejected this assumption, there were variations in BMI growth patterns i.e. there were different adiposity trajectories, and these patterns differed between boys and girls between 5 to 18 years of age. Hence, I concluded that heterogeneity in adiposity trajectories do exist from 5 to 18 years of age in black South African children.

6.3.2 Hypothesis 1.3.2.i: There are no differences in relationships between childhood adiposity trajectories and elevated and obesity risk later in life.

I have also rejected this hypothesis since children that followed adiposity trajectories characterized by high BMI as early as 5 years and remain so up to 18 years had a higher risk of elevated BP at 18 years and higher obesity risk at 23 years compared to their pears in normal weight trajectory.

6.3.3 Hypothesis 1.3.2.ii: Birth weight, early life weight gain and genetics are not predictors of BMI trajectory membership.

Conditional weight gain in both infancy and mid-childhood was significantly associated with early onset of obesity or overweight BMI trajectories. A higher genetic risk score increased individuals to follow an obese BMI trajectory membership. I rejected the influence of birth weight on adiposity trajectories membership in the current study. I concluded that rapid early life growth and genetics were predictors of adiposity trajectories.

6.3.4 Hypothesis 1.3.2.iii: A genetic risk score has no effect on BMI through infancy and adolescence.

The genetic risk score was not associated with BMI from birth to mid childhood hence this assumption did not hold. But it had an influence on BMI from mid-childhood to late adolescence.

6.3.5 Hypothesis 1.3.2.iv: Both childhood and adolescent growth have mediation role on genetic influence on adult BMI but infancy had no such influence.

6.4 Emerging research themes from the findings

6.4.1 Latent class models

South Africa, like other low and middle-income countries (LMICs), is faced with a high burden of chronic disease with 70% and 25% of women and children being overweight or obese respectively. Identifying and understanding the factors that influence life-course overweight and obesity from childhood to adolescence and the associated future health risk factors are of paramount importance. Categorization has played a vital role in epidemiology, for example, categorizing weight status into normal, overweight, obese and morbidly obese help the researchers or clinicians make better decisions on who should be targeted for an intervention or for further treatment (Hoekstra, 2013). Grouping individuals based on their developmental trajectories of a particular risk factors helps to better understand the aetiology of the disease (onset and progression) and its relationship with risk factors. The LCGMM used in this thesis helped to identify those children (subgroups) in high-risk trajectories. In LCGMM, the at high-risk trajectories are usually the smallest, 1.4 % for boys and 4.5% for girls in our case. Using common statistical approaches such as mixed models (Twisk, 2006) and generalized equation (Twisk, 2013) could have not taken into consideration these unobserved high risk groups.

Advantages

Firstly, identification of trajectories is not based on predefined categories, but on available data. Using LCGMM in identifying trajectories is an ideal analytical method since it involves a number of criteria in selecting the best fitting model and it predicts individual class membership from the available data and its ability to deal with missing at random data. Latent class is focused on relationships among individuals, putting those with similar developmental patterns in the same subgroup (Berlin, Parra, et al., 2014; Hoeksma et al., 2006; Jung et al., 2008; Muthen, 2001; Nagin, 2005). The subgroups identified from LCGMM represent different categories of individuals e.g. individuals with different disease onset and progression. Lastly LCGMM acknowledges heterogeneity in the study population and summarizes individuals within the subgroup. Unlike the one-size fits all where the assumption is that only one developmental trajectory exist and the average for the whole population is used.

Latent class models have the potential to be used more in epidemiology and genetics, for example in Randomized Clinical Trial (RCT). The main purpose in RCT is to estimate treatment or intervention effect. Using LCGMM can help to check the effectiveness of a particular treatment or intervention within the identified subgroups. Latent class models can also be used to assess the variations in interventions effects in longitudinal trials. This would help the researcher to establish when the intervention is more effective, hence helping in future intervention strategies and even to employ appropriate strategy for specific subgroups. The latent class models can also help to understand which treatment option works better for which particular subgroup and when, based on the trajectory membership. This would help in the current concept of personalized medicine.

In genetics, the use of latent class analysis could help to find the genetic risk factors associated with different stages of disease onset or progression. It would also help to unearth those longitudinal genetic effects of complex traits. Researchers would be able to find which genes, SNPs or alleles are associated with a particular subgroup and why. In doing so it would help in doing a genetic risk profiling of individuals. Using latent class models would also allow harmonization between studies that collected data at different time points. These studies would be merged using the trajectory membership, putting individuals with similar trajectories together. The latent class membership can also be used as quantitative traits in a GWAS and other genetic studies.

Limitations

The use of LCGMM has been critically reviewed in epidemiology and other fields (Bauer, 2007; Bauer et al., 2003; Berlin, Parra, et al., 2014; Hoeksma et al., 2006; Hoekstra et al., 2011; Jung et al., 2008; Londono et al., 2013; Muthen, 2001; Nagin, 2005; Nylund et al., 2007). The complexity on one hand and the flexibility on the other of latent class models result in challenges around computation power and choosing the optimal number of classes. When I tried to model BMI trajectories with several covariates (both time varying and non-time varying) in Mplus which implements LCGMM, resulted in converging problems and a long time to compute (in some cases a few days). The other complexity comes in choosing optimal number of latent classes. There are a number of model fit statistics choices that a researcher can look at when choosing optimal number of classes. Sometimes these model statistics give inconsistent results as such the decision depends on the researcher's choice. There are still no clear guidelines in choosing the optimal number of classes. BLRT has been recommended to be the most consistent model fit index but researchers still struggle with this problem in practice (McLachlan et al., 2004; Nylund et al., 2007). The decision taken by the researcher at this point will affect both the number of trajectories and the number of individuals in those trajectories.

This would have an influence on further analyses and interpretation of the results more than the decisions taken when in a standard regression analysis.

Another limitation is related to which latent classes model to use. In LCGA the within class-variance parameters are fixed to zero, meaning that the individuals in the same subgroup have the same intercept and slope, while in LCGMM the within-class variance parameters are freely estimated hence there is a within-class variations in growth parameters (i.e. intercept and slope). Applying LCGA will end up with more latent classes than using LCGMM (Berlin, Williams, et al., 2014; Berlin, Parra, et al., 2014; Hoekstra, 2013; Hoekstra et al., 2011; Jung et al., 2008; Muthen, 2001; Nagin, 2005). The decision on whether to use LCGA or LCGMM will depend on the research question to be answered, the framework and the intended purpose of the identified trajectories. If the research question focuses on the individual variation of BMI developmental patterns in subgroups in a population and describing the identified patterns then LCGMM would be the ideal approach since it allows for both between and within classes variation in growth parameters. If the aim is to determine early factors or future risk associated with particular subgroup then the within-class variation can be relaxed and LCGA can be the referred approach. However, if the research question considers both then LCGMM should be considered. Lastly the latent class approaches are also affected by design issues such as sample size and number of

repeated measures available per an individual. These also could affect the number of trajectories being identified (Connell et al., 2006; Hoeksma et al., 2006; Hoekstra, 2013).

Despite all these issues the results from this study are robust as consistent results were observed different model fit statistics criteria for choosing optimal number of latent classes. No convergence problems were observed due to small sample sizes were observed and with an average of eight repeated measures per individual increased the precision of the predicted models.

Other issues

There are limited resources on application of latent class models in epidemiology and they are hardly used in genetics studies. There is a need for more tutorial and proper guidelines on how to implement these models. The other issue that may contribute slow penetration on the use of latent class models in epidemiology and genetics was that all the software that implement these models such as Mplus (Muthén & Muthén, Los Angeles, USA), SAS (SAS Institute Inc., Cary, North Carolina, USA), STATA (StataCorp, College Station, TX, USA) and LatentGOLD (Statistical Innovations, Belmont MA, USA), are proprietary software with some costing over $1000, which is expensive to most researchers in LMICs. I could not find any reliable open source software that implements LCGMM.

6.4.2 Heterogeneity in BMI growth patterns and future health risk

Most obesity interventions programmes have been at population level with an assumption of one size fits all principle, where it is assumed that all individuals in the population follow the same growth curve with same slope and intercept, but through this study, I have learnt that there is heterogeneity in the development of BMI overtime between in individuals. There is variation in number, shape and membership (%) of adiposity trajectories, these variations were also observed between boys and girls. Taking advantage of LCGMM to explore the variation of BMI developmental patterns, I was able to identify these BMI subgroups from 5 to 18 years old. With the advent of personalized public health concept, this approach would be helpful to design more efficient treatment and prevention strategies, taking into account that the population level interventions on adult BMI have not been as effective. It calls for differentiated prevention and intervention strategies for individuals belonging to each of the identified adiposity trajectories (Larsen et al., 2017).

One of the striking results was the discovery of an obese subgroup as early as five years. Such a subgroup would be the one of much interest when coming up intervention strategies since childhood obesity has negative health outcomes both during childhood and later in life. These intervention programmes would be targeted depending on the adiposity trajectories since the BMI development would vary, which would also impact the health risks an individual would be exposed to later in life.

In empirical chapters four and five I discuss the factors that would drive an individual to follow a particular adiposity trajectory membership. I discussed that conditional relative weight gain in infancy and early childhood and a higher genetic risk score for BMI increases the odds of an individual to belong to an early obese/overweight adiposity trajectories. I also discussed that girls are at higher risk of transitioning into an obese trajectory, as they get older compared to boys. In this study birth weight and maternal height were not predictors of adiposity trajectory membership, which is in contrast to some previous studies in Caucasian (European, Australian children) population (Brault et al., 2015; Li et al., 2007; Magee et al., 2013; Nonnemaker et al., 2009; Pryor et al., 2011). Understanding the pathways involved in the faster weight gain and genes involved in childhood adiposity would help to delay individuals to follow obese adiposity trajectories or even change the course of BMI growth pattern to the normal adiposity trajectory.

Existing literature has shown that childhood overweight or obesity is linked to different risky health outcomes in adulthood but the unanswered has been when in childhood would be the best indicator of such risk. I found that boys and girls belonging to adiposity trajectories characterized by accelerated BMI from five years through to late adolescence had higher odds of being obese and have elevated BP in young adulthood compared to those in a normal weight adiposity trajectory. This would mean that the adiposity trajectory membership would help us to identify individuals at risk of certain undesirable health outcomes later in life, hence implementing interventions that would change an individuals course of the trajectory to follow earlier in life would be of much benefit to that individuals health later in life.

I found that the genetic influence on BMI became more relevant in the teenage (from 11 years). Conditional relative weight gain during this period mediated this influence. The genetic influence was not significant in infancy and childhood. The genetic risk of obesity in late adolescence (at 18 years old) was mediated by relative conditional weight gain pre-and during adolescence. From the findings, the use of genetic risk score gives us an overall genetic effect, which is independent of

other factors such as environment. The mediation analysis also opens a way to do more focused studies involving the individual genes to test for causality.

6.5 Strengths and limitations

6.5.1 Strengths

The prospective design nature of the study gives a chance for long term follow up of individuals from birth to 23 years, which puts this study a potential to study causality. The sample size was large, the longitudinal aspect of the study and the analytical variables for excluded and included samples were compared which increased external validity. The same validated questionnaires for SES and anthropometry were used over the study period. The cohort had repeated measures for BMI from birth to 23 years and trained research assistants collected BP and anthropometric measures. Analyses were adjusted for different confounders, which were measured at different data collection periods hence increasing the potential of establishing causality from this study. The exploratory analyses to check the patterns of missing data on weight and height concluded that data was missing at random. To distinguish between individuals with different BMI developmental trajectories, we utilized LCGMM to identify BMI trajectories which is an ideal analytical method since it involves a number of criteria in selecting the best fitting model and it predicts individual class membership based on the available data. LCGMM also has the ability to deal with missing data with the missing at random assumption. From literature and to my knowledge, this study is one of only a few that focuses on an African cohort to use LCGMM hence it provides a unique, in-depth understanding of BMI trajectories, early life factors associated, future health risk associated and genetic effect on both the trajectories and longitudinal BMI in South Africa. The use of a genetic risk score increased the power of the analysis. Genetic associations using wGRS are unlikely to be affected by potential confounding factors such as age and environment. The use of different longitudinal methods to understand the influence of the genetic risk score on BMI suggested the likely utility of modelling BMI trajectories in future genetic studies in large sample sizes. This approach would also allow harmonization between studies that collected data at different time points.

The use of different longitudinal methods to understand the influence of the genetic risk score on BMI suggested the likely utility of modelling BMI trajectories in future genetic studies in large sample sizes. This approach would also allow harmonization between studies that collected data at different time points.

The results from this thesis underscore the importance of studying individual's BMI developmental patterns instead of looking at the development of BMI at population level. Characterizing and understanding trajectory groups of children who are likely to become obese or overweight as they transition from childhood to adolescence then to adulthood is critical for intervention strategies and health services. This puts forward the need for identifying such individuals who are in high risk BMI trajectories as a form of screening and the interventions or education programmes should target such groups at early stage hence reduce the adverse health risk outcomes later in life.

The results from BMI trajectories and genetic risk score showed that individuals with genetic susceptibility of obesity with interaction of unhealthy living have a higher risk of developing obesity at earlier stage. The fact that the strongest associations were observed during adolescence which may be explained in terms of anatomical and physiological changes in response to environment conditions during such critical or sensitive periods of development. Using trajectories can help to find these turning point periods where an individual move from one trajectory membership to another. It may also signify the interactions between genetics and environment at these crucial periods.

6.5.2 Limitations

Firstly, we observed an overall attrition of about 25%, loss due to follow up, in this cohort, this was inevitable due to deaths, migrations and other reasons. The trajectories characterized by early onset obese or overweight comprised of small number of individuals that could affect the association analyses in this study. Maternal factors that could have influenced the outcomes in this study, such as smoking and alcohol consumption before and during pregnancy, were not available in this study, and are a noted limitation.

It should be noted that at this stage it is not possible to determine whether the early life growth risk factors assessed in this study are causally involved in obesity development, which highlights the importance of further investigation to fully elucidate causality through techniques such as Mendelian randomization.

The genetic risk score approach gives us an insight into the overall genetic susceptibility but much larger studies will be required to examine effects of specific individual genes. We weighted the GRS based on effect sizes reported in Caucasian populations, which might have different size effects in African populations and could not use the Metabochip data to explore novel genetic variants of obesity.

The use of BMI as an obesity or overweight measure is common but it is not a reliable measure for fatness, need to use DXA data (body composition, central adiposity measures), for instance BMI cannot distinguish between someone who is muscular with strong bones and little fat from someone with a lot of fat. We did not involve diet and physical activities data; future research should take these into consideration.

6.6 Future research

After this study, the following possible future research gaps are noted:

1. Classify/screen individuals based on genetic risk score and BMI trajectory membership to identify those at high-risk individuals and relate to future risks. This would help when coming up with prevention and intervention strategies for obesity based on the trajectory membership and the value of the genetic risk. Those individuals in early onset obesity and at the same time having a higher value of obesity risk score might need immediate attention.
2. Identify from childhood to adulthood BMI trajectories and use them as a quantitative trait in a GWAS to find which genetic markers are associated with specific BMI trajectories. This would help to identify genetic variants that are associated with a trait or disease at specific stage e.g. those, which are associated with early onset or late onset or progression or the entire period.
3. Use large scale GWAS data in African populations and do meta-analysis of GWAS of obesity in Africa, use the effect sizes from these analyses to construct a BMI GRS.
4. Since genetic risk variants require a specific environment to trigger its effect, there is a need for large-scale longitudinal studies involving gene-gene and gene-environment interactions, which would also help to infer causality of the observed associations.

6.7 Implications

The knowledge gained from the findings of this thesis would help public health practitioners, clinicians and policy makers in designing interventions, prevention or education programmes to reduce public health burden of obesity from childhood in a population. One way is to come up with evidence-based public health strategies that focus and monitor early childhood growth development, based on adiposity trajectory membership and genetic risk score value, so as to deal with deleterious effects on future health, social and economic outcomes in adulthood. These

programmes should target individuals at risk at early stage hence reduce the adverse health risk outcomes later in life.

6.8 Conclusions

In conclusion, this thesis has demonstrated the importance of investigating life-course variation in childhood adiposity development. It has demonstrated that early life conditional relative weight gain and a higher genetic risk score accelerated individuals to be in or increased the risk of transitioning into an obese trajectory. It also highlights the future risks associated with the unfavorable adiposity trajectories, for instance, children who were overweight or obese in childhood remained so until late adolescence and were high at risk of elevated BP in late adolescence.

It has also highlighted that the variants from the recent GWAS, predominantly from Caucasian population, had cumulative effects on longitudinal BMI and there was a synergistic effect of a high genetic risk score of the recent adult BMI-associated SNPS on the obese phenotype from mid-childhood to late adolescence. The genetic predisposition to increased BMI in late adolescence was attenuated by rapid conditional weight gain pre-and during adolescence in this South African population.

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Appendices

Appendix 1: Details of the 97 reported BMI genome wide loci. Source: (Locke et al., 2015)

SNP	Chromosome	Position (bp)	Nearest gene
rs977747	1	47,457,264	*TAL1*
rs657452	1	49,362,434	*AGBL4*
rs11583200	1	50,332,407	*ELAVL4*
rs3101336	1	72,523,773	*NEGR1*
rs12566985	1	74,774,781	*FPGT-TNNI3K*
rs12401738	1	78,219,349	*FUBP1*
rs11165643	1	96,696,685	*PTBP2*
rs17024393	1	109,956,211	*GNAT2*
rs543874	1	176,156,103	*SEC16B*
rs2820292	1	200,050,910	*NAV1*
rs13021737	2	622,348	*TMEM18*
rs10182181	2	25,003,800	*ADCY3*
rs11126666	2	26,782,315	*KCNK3*
rs1016287	2	59,159,129	*LINC01122*
rs11688816	2	62,906,552	*EHBP1*
rs2121279	2	142,759,755	*LRP1B*
rs1460676	2	164,275,935	*FIGN*
rs1528435	2	181,259,207	*UBE2E3*
rs17203016	2	207,963,763	*CREB1*
rs7599312	2	213,121,476	*ERBB4*
rs492400	2	219,057,996	*USP37*
rs2176040	2	226,801,046	*LOC646736*
rs6804842	3	25,081,441	*RARB*
rs2365389	3	61,211,502	*FHIT*
rs3849570	3	81,874,802	*GBE1*
rs13078960	3	85,890,280	*CADM2*
rs16851483	3	142,758,126	*RASA2*
rs1516725	3	187,306,698	*ETV5*
rs10938397	4	44,877,284	*GNPDA2*
rs17001654	4	77,348,592	*SCARB2*
rs13107325	4	103,407,732	*SLC39A8*
rs11727676	4	145,878,514	*HHIP*

rs2112347	5	75,050,998	*POC5*
rs7715256	5	153,518,086	*GALNT10*
rs205262	6	34,671,142	*C6orf106*
rs2033529	6	40,456,631	*TDRG1*
rs2207139	6	50,953,449	*TFAP2B*
rs9400239	6	109,084,356	*FOXO3*
rs9374842	6	120,227,364	*LOC285762*
rs13201877	6	137,717,234	*IFNGR1*
rs13191362	6	162,953,340	*PARK2*
rs1167827	7	75,001,105	*HIP1*
rs2245368	7	76,446,079	*PMS2L11*
rs9641123	7	93,035,668	*CALCR*
rs6465468	7	95,007,450	*ASB4*
rs17405819	8	76,969,139	*HNF4G*
rs16907751	8	81,538,012	*ZBTB10*
rs2033732	8	85,242,264	*RALYL*
rs4740619	9	15,624,326	*C9orf93*
rs10968576	9	28,404,339	*LINGO2*
rs6477694	9	110,972,163	*EPB41L4B*
rs1928295	9	119,418,304	*TLR4*
rs10733682	9	128,500,735	*LMX1B*
rs7899106	10	87,400,884	*GRID1*
rs17094222	10	102,385,430	*HIF1AN*
rs11191560	10	104,859,028	*NT5C2*
rs7903146	10	114,748,339	*TCF7L2*
rs4256980	11	8,630,515	*TRIM66*
rs11030104	11	27,641,093	*BDNF*
rs2176598	11	43,820,854	*HSD17B12*
rs3817334	11	47,607,569	*MTCH2*
rs12286929	11	114,527,614	*CADM1*
rs7138803	12	48,533,735	*BCDIN3D*
rs11057405	12	121,347,850	*CLIP1*
rs12016871	13	26,915,782	*MTIF3*
rs12429545	13	53,000,207	*OLFM4*
rs9540493	13	65,103,705	*MIR548X2*
rs1441264	13	78,478,920	*MIR548A2*
rs10132280	14	24,998,019	*STXBP6*

rs12885454	14	28,806,589	*PRKD1*
rs11847697	14	29,584,863	*PRKD1*
rs7141420	14	78,969,207	*NRXN3*
rs3736485	15	49,535,902	*DMXL2*
rs16951275	15	65,864,222	*MAP2K5*
rs7164727	15	70,881,044	*LOC100287559*
rs758747	16	3,567,359	*NLRC3*
rs12446632	16	19,842,890	*GPRC5B*
rs2650492	16	28,240,912	*SBK1*
rs3888190	16	28,796,987	*ATP2A1*
rs4787491	16	29,922,838	*INO80E*
rs9925964	16	31,037,396	*KAT8*
rs2080454	16	47,620,091	*CBLN1*
rs1558902	16	52,361,075	*FTO*
rs9914578	17	1,951,886	*SMG6*
rs1000940	17	5,223,976	*RABEP1*
rs12940622	17	76,230,166	*RPTOR*
rs1808579	18	19,358,886	*C18orf8*
rs7239883	18	38,401,669	*LOC284260*
rs7243357	18	55,034,299	*GRP*
rs6567160	18	55,980,115	*MC4R*
rs17724992	19	18,315,825	*PGPEP1*
rs29941	19	39,001,372	*KCTD15*
rs2075650	19	50,087,459	*TOMM40*
rs2287019	19	50,894,012	*QPCTL*
rs3810291	19	52,260,843	*ZC3H4*
rs6091540	20	50,521,269	*ZFP64*
rs2836754	21	39,213,610	*ETS2*

R14/49 Mr Richard Munthali

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M1411115

NAME: (Principal Investigator)	Mr Richard Munthali
DEPARTMENT:	Molecular and Cell Biology Sydney Brenner Institute for Molecular Bioscience
PROJECT TITLE:	Longitudinal Analysis of Genotype-Phenotype Associations in Obesity and Elavated Blood Pressure in the Black South African Population
DATE CONSIDERED:	Adhoc
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Zane Lombard
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	12/01/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature — Date: 12/01/2015

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 2: Ethics clearance for the reuse of human data

Munthali *et al. BMC Public Health* (2016) 16:665
DOI 10.1186/s12889-016-3337-x

BMC Public Health

RESEARCH ARTICLE Open Access

Childhood adiposity trajectories are associated with late adolescent blood pressure: birth to twenty cohort

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Abstract

Background: Elevated blood pressure in childhood is a risk factor for adult hypertension which is a global health problem. Excess adiposity in childhood creates a predisposition to develop adult hypertension. Our aim was to explore distinct sex-specific adiposity trajectories from childhood to late adolescence and examined their association with blood pressure.

Methods: Latent Class Growth Mixture Modeling (LCGMM) on longitudinal data was used to derive sex-specific and distinct body mass index (BMI: kg/m^2) trajectories. We studied 1824 black children (boys = 877, girls = 947) from the Birth to Twenty (Bt20) cohort from Soweto, South Africa, and obtained BMI measures at ages 5 through 18 years. Participants with at least two age-point BMI measures, were included in the analysis. Analysis of variance (ANOVA), chi-square test, multivariate linear and standard logistic regressions were used to test study characteristics and different associations.

Results: We identified three (3) and four (4) distinct BMI trajectories in boys and girls, respectively. The overall prevalence of elevated blood pressure (BP) was 34.9 % (39.4 % in boys and 30.38 % in girls). Boys and girls in the early onset obesity or overweight BMI trajectories were more likely to have higher BP values in late adolescence. Compared to those in the normal weight BMI trajectory, girls in early onset obesity trajectories had an increased risk of elevated BP with odds ratio (OR) of 2.18 (95 % confidence interval 1.31 to 4.20) and 1.95 (1.01 to 3.77). We also observed the weak association for boys in early onset overweight trajectory, (p-value = 0.18 and odds ratio of 2. 39 (0.67 to 8.57))

Conclusions: Distinct weight trajectories are observed in black South African children from as early as 5 years. Early onset adiposity trajectories are associated with elevated BP in both boys and girls. It is important to consider individual patterns of early-life BMI development, so that intervention strategies can be targeted to at-risk individuals.

Keywords: Blood pressure, Latent classes, Latent class growth mixture modeling, Body mass index trajectories, Trajectories, Hypertension, Childhood adiposity, Obesity

Background

Hypertension is a global public health problem affecting more than one billion people globally [1]. It is a risk factor for developing cardiovascular disease (CVD) and also contributes to an increase in mortality worldwide. A recent systematic analysis reported that hypertension accounts for about nine million deaths globally every year [2]. In African countries, 25 % of deaths are caused by hypertension [1]. Hypertension is also one of the leading causes of heart attacks, stroke and kidney failure [2]. Results from the Heart of Soweto Study reported a 55 % hypertension prevalence among adult South Africans aged 52.8 years on average [3]. Some studies have looked at elevated blood pressure (BP) in childhood, mostly in rural South Africa, with reported prevalence rates ranging from 1 to 25.9 % [4–7].

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Previous studies have reported that childhood blood pressure weight gain from childhood to adulthood, adulthood obesity [8, 9], childhood and adolescent physical in-activity and certain lifestyle behaviors such as use of tobacco and alcohol consumption [10], are some of the determinant factors of adulthood hypertension. Understanding the life-course progression of adiposity in children is important since childhood adiposity is associated with adult obesity which has been reported to be linked to increased hypertension risk in adults [11]. Kagura and colleagues reported that elevated BP can be observed from childhood in black urban South African children [12]. Most of the studies that reported on childhood obesity and adult hypertension have used cross sectional data. There are few studies that have used longitudinal data to understand the effect of life-course childhood adiposity on late adolescent blood pressure and lack of comprehensive longitudinal data have made it difficult to do such studies in an African setting.

There has been increased interest in the role of growth patterns on BP, it is also still unclear how early childhood adiposity influences later BP [13]. Using longitudinal data will help elucidate this relationship and this study in particular will add further evidence from an African perspective. For the first time in an exclusively African population, we sought to determine whether adiposity progression (from 5 to 18 years of age), focused on using body mass index (BMI) as an adiposity marker, could be used to predict BP among late adolescents.

We use a relatively new method in both biology and epidemiology called Latent Class Growth Mixture Modeling (LCGMM) to identify distinct sex-specific adiposity trajectories. LCGMM groups individuals with the same developmental trajectory in the same class. It also allows for inclusion of variation in several growth parameters both within and between classes [14]. Developmental trajectories of BMI describe individuals' BMI change over time. One of the advantages for using LCGMM is that adiposity trajectories are identified independently without pre-assumptions of the relative contributions of various lifestyles, genetic and epigenetic factors. Therefore multiple factors may contribute to variation in the information of these adiposity trajectories.

The aims of this study were to: 1) determine the distinct sex-specific patterns of adiposity trajectories in black South Africa children from 5 to 18 years of age, 2) find the prevalence of elevated blood pressure in late adolescence, and 3) to explore associations between these distinct adiposity trajectories to elevated BP in late adolescence.

Methods

Participants

To identify developmental patterns of BMI from 5 to 18 years old and relate them to blood pressure in late adolescence, data from the Birth to Twenty cohort (Bt20) were used. Bt20 is Africa's largest and longest running longitudinal birth cohort, with 3273 children at time of enrolment. It is focused on the health and development of children born in a South African urban township, namely Soweto in Johannesburg. The cohort comprised of 1682 girls and 1591 boys of which the majority were black children (78.4 %). The participants have been followed up to date using different means of communication. The detailed cohort profile with recruitment and cohort attrition details has been described elsewhere [15]. Only black participants ($n = 1824$) with weight and height data available for at least two time points between 5 and 18 years were included in this study.

Measures

Anthropometrics

Trained research assistants collected anthropometric measurements. Weight was measured using a digital scale to the nearest 0.1 kg with participants in light clothes and without shoes. A wall-mounted stadiometer (Holtain, UK) was used to measure standing height to the nearest 0.1 cm. Weight and height at 5 years and 7–18 years old were used to calculate BMI (weight (kg)/height (m^2)), used as a marker for adiposity at corresponding years.

Blood pressure assessment

Blood pressure (mm Hg) was measured using Omron 6 automated machine (Kyoto, Japan) from 8 years of age onwards and a Dinamap Vital Signs monitor 1846SX (Critikon, USA) was used at 5 years of age. At each assessment, participants' seated blood pressure was measured three times with a 2 min interval between each measurement. The blood pressure measurements were taken after 5 min of seated rest. The mean average for the second and third right arm readings was recorded for the current analysis. Blood pressure measurements from one visit have been used in blood pressure studies before [12, 13, 16, 17].

The mean arterial pressure (MAP) was calculated from systolic blood pressure (SBP) and diastolic blood pressure (DBP) using the formula; MAP = (SBP + (2 * DBP))/3. We followed a standard procedure as recommended by the fourth National High Blood Pressure Education Program working group on high blood pressure in children and adolescents (NHBPEP) [18] report to classify blood pressure either normotensive (BP less than 90th percentile), prehypertension or hypertension (BP equal or over 90th percentile for sex, age and height) for those participants who were above 17 years but not yet 18 years old the time BP data was collected. For participants who were already 18 years or older we used the cut-offs as recommended in the seventh report of the Joint National Committee on

Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [19]. Prehypertension was defined as SBP readings from 120 to 139 mm Hg or a DBP from 80 to 89 mm Hg while hypertensive was defined as SBP readings equal or greater than 140 mm Hg or DBP readings equal or greater than 90 mm Hg. Due to small sample size the prevalence of hypertension, prehypertension and hypertension were combined and defined as elevated blood pressure.

Statistical analysis

Different group based modeling methods such as Latent Class Growth Analysis (LCGA) and Latent Class Growth Mixture Modeling (LCGMM), have been used to identify and classify individuals into different trajectories. LCGA technique was developed by Nagin and colleagues [20–23] and is implemented through SAS Proc Traj in the SAS software. There is also a Traj Stata software plugin developed by Jones and Nagin, 2012 [24]. On the other hand LCGMM was developed by Muthén and colleagues and is implemented in Mplus [25–28]. We used Mplus and LCGMM since it allows variation in the intercept and slope in both within the class (inter-individual differences) and across classes while SAS Proc Traj allows only across classes variation. Using LCGMM adds more heterogeneity in the model, thus being the more flexible method as such was preferred in the current analysis [14, 22, 25, 26, 28–30].

The exploratory analyses to check the patterns of missing data concluded that data was missing at random. Mplus handles missing data by the expectation-maximization algorithm (EM algorithm) with assumption of missing at random (MAR) [28]. This makes use of full information maximum likelihood estimation with missing values (FIML) by using estimation to integrate all available information based on MAR assumptions. This means that we can make full use of all available data in our analysis. This prevents the inclusion of only those participants who have no missing data at all data points, which would subsequently reduce the sample size. So the use of the maximum likelihood (ML) approach implemented in Mplus overcomes potential biases in participants with substantial missing data [25, 26, 28, 31, 32].

We included only those participants with at least two time points of available data for BMI in the current analysis. Those with one time point of available data were excluded as this may have influenced the identification and pattern of the BMI trajectories. On average, seven available data points per participant were used to perform this longitudinal analysis.

Latent Class Growth mixture modeling (LCGMM in Mplus version 7.3 [28] was performed to identify the distinct sex-specific BMI trajectories between 5 and 18 years.

The BMI values were used in this study because they are more sensitive to changes in body composition and have been recommended over BMI z-scores [33–35].

Once BMI trajectory group membership was determined for both girls and boys, each respondent was assigned to the class according to the highest probability of belonging to that class. The remainder of analyses was conducted using Stata version 13 [36] to examine additional descriptive characteristics of the classes. We used both standard logistic and multivariate linear regressions to estimate the relationship between latent class membership and blood pressure in late adolescence. Analysis of variance (ANOVA) and chi-square test results were used to assess the differences in different study characteristics among the BMI trajectory classes at 5 % level of significance.

Results

BMI Trajectories: optimal number of BMI trajectories

Using LCGMM, we explored linear, quadratic and cubic slopes to fit the model. The cubic slope coefficients were either not significant or required prohibitive running times and did not converge in all models. This suggests that the cubic time function model did not fit the data well. After applying the criteria as recommended by other authors [14, 25, 26, 29, 30, 37–39], only quadratic models involving quadratic time function were further analyzed.

To determine the optimal number of latent classes, we used different model fit indices in conjunction with other criteria as used in previous studies [14, 30, 37, 40, 41]. Firstly, we looked at the three model statistics, the Bayesian Information Criterion (BIC) [42], the Bootstrap Likelihood Ratio Test (BLRT) [43] and Lo, Mendell and Rubin Likelihood Ratio Test (LMR-LRT) [44]. Some studies have applied BIC in variable modeling analyses [41, 42, 45, 46]. It estimates a model to be true using posterior probabilities. A lower BIC would indicate that a model is more likely to be considered as the true model [42]. BIC reduces the false positive rate, hence most individuals will be assigned in their right BMI latent class [47]. We also looked at the entropy value and number of study participants per class. We employed a less restrictive 1 % group membership in this study, which has also been used before in other studies [41, 48]. Lastly we looked at the shape of the BMI trajectories and its clinical interpretability.

Model fit statistics used to come up with optimal number of classes in both boys and girls are shown in Table 1. Applying the criterion discussed above a four-class model fits our data well in girls and a three-class solution has the best classification of BMI trajectory membership in boys.

In order to facilitate interpretation of the results, Fig. 1a and b show the BMI trajectory classes for 947 girls and 877 boys in relation to the extended International

Table 1 Model fit statistics for quadratic LCGMM comparing solutions for 1 to 7 latent classes

Sex	Latent classes	BIC[a]	LMR-LRT[b] (*P*-value)	BLRT[c] (*P*-value)	Convergence	Entropy value	Log Likelihood	Number of parameters
Boys	1	23605.4	N/A	N/A	Yes	N/A	−11738.3	19
	2	22846.6	0.03	0.00	Yes	0.98	−11345.4	23
	3[d]	**22644.4**	**0.13**	**0.67**	**Yes**	**0.99**	**−11230.7**	**27**
	4	22544.7	0.53	1.00	Yes	0.98	−11167.3	31
	5	22476.5	0.77	0.67	Yes	0.93	−11119.7	35
	6	22438.4	0.61	1.00	Yes	0.94	−11087.1	39
	7				NO			43
Girls	1	27949.8	N/A	N/A	Yes	N/A	−13909.8	19
	2	27226.2	0.00	0.00	Yes	0.94	−13534.3	23
	3	27155.0	0.10	0.00	Yes	0.76	−13485.0	27
	4[d]	**27083.9**	**0.23**	**0.00**	**Yes**	**0.80**	**−13435.7**	**31**
	5	27026.0	0.45	0.6	Yes	0.82	−13393.1	35
	6	26990.9	0.63	1.00	Yes	0.78	−13361.8	39
	7	26961.2	0.27	1.00	Yes	0.80	−13333.3	43

LCGMM latent class growth mixture modeling
[a]Bayesian Information Criterion
[b]Lo, Mendell and Rubin Likelihood Ratio Test
[c]Bootstrapped Likelihood Ratio Test, N/A – not applicable
[d]The optimal class solutions according to the model fit criteria are shown in bold

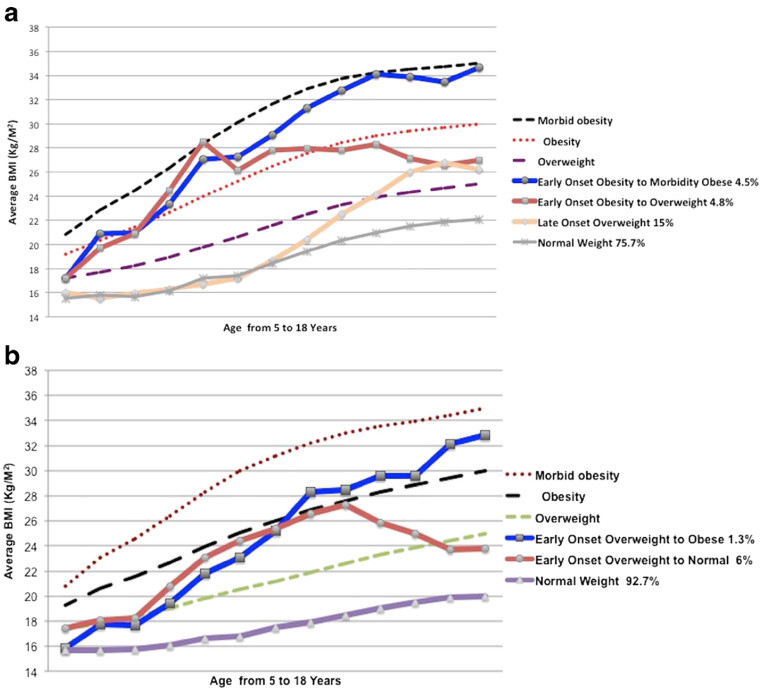

Fig. 1 **a** BMI Trajectories in girls. BMI latent classes plotted together with Extended International Obesity Task Force (IOTF) Cut-Offs for BMI in girls. **b** BMI Trajectories in boys. BMI latent classes plotted together with Extended International Obesity Task Force (IOTF) Cut-Offs for BMI in boys

Obesity Task Force (IOFT) [49]. For girls, four BMI trajectory classes can be described as: Trajectory 1 (normal weight: 75.7 %), Trajectory 2 (late onset overweight: 15 %), Trajectory 3 (early onset obesity to overweight: 4.8 %) and lastly Trajectory 4 (early onset obesity to morbidly obese: 4.5 %). For boys, a three-class model fits the data well. These classes can be classified as: Trajectory 1 (normal weight: 92.7 %), Trajectory 2 (early onset overweight to normal: 6 %) and Trajectory 3 (the early onset overweight to obese trajectory: 1.3 %).

Study characteristics by BMI trajectory classes are shown in Tables 2 and 3. Most of the study characteristics were significantly different among BMI trajectories in girls. In boys, lower maternal education and SBP at 18 years were significantly different among the BMI trajectories and the rest were not.

Blood pressure in late adolescence and BMI trajectory classes

Only participants with blood pressure values at 18 years of age were included in this analysis. It included 766 boys and 823 girls. The overall prevalence of elevated blood pressure in late adolescence was 34.91 % (39.43 % boys and 30.38 % girls). Birth weight and height at 18 years were added in the model to determine whether BMI trajectory classes have an effect on SBP, DBP, MAP and elevated BP status as shown in Tables 4 and 5. In girls, Trajectory 3 was significantly associated with SBP, DBP and MAP with mean BP (95 % confidence interval, 95 % CI) of 4.18 mm Hg (1.03–7.33), 4.44 mm Hg (1.65 to 7.22) and 4.35 mm Hg (1.70 to 7.01) compared to normal trajectory, respectively. Trajectory 4 was significantly associated with SBP, DBP and MAP with mean BP of 6.08 mm Hg (2.93 to 9.23), 4.28 mm Hg (1.50 to 7.07) and 4.88 mm Hg (2.23 to 7.54) compared to normal weight trajectory, accordingly. Girls in Trajectories 3 and 4 had a 1.95 fold (1.01 to 3.77) and a 2.18 fold (1.31 to 4.20) increased risk of elevated BP in late adolescence, separately.

For boys, Trajectory 3 was significantly associated with SBP, DBP and MAP with mean elevated BP of 13.45 mm Hg (6.82 to 20.07), 5.74 mm Hg (0.30 to 11.10) and 8.31 mm Hg (3.30–13.32) compared to normal weight trajectory respectively. In boys we observed a weak association for Trajectory 3 with a 2.39 fold (0.67 to 8.57) increased risk of elevated BP in late adolescence. The main results did not vary adjusting by birth weight, height at 18 years or both in girls. In boys, only the association between Trajectory 3 and DBP varied after adjusting by both weight and height at 18 years. The association was not significant in the unadjusted model while significant in the adjusted model.

Discussion

There has been a growing interest in studying the heterogeneity in adiposity (BMI) developmental patterns in different populations over past years. Taking advantage of data-driven methods such as LCGMM we explored the distinct developmental patterns of BMI across childhood to late adolescence and its association with late adolescent blood pressure in black South African children. Our results confirm that there is heterogeneity in BMI trajectories in the study sample and that trajectories vary between boys and girls. A four-class BMI trajectory model may best represent heterogeneity in BMI developmental patterns in girls (normal weight, late onset overweight, early onset obesity to overweight and early onset obesity to morbidly obese) and a three-class model among boys (normal weight, early onset overweight to normal and early onset overweight to obese trajectory). These results are consistent with those reported by Hejazi and colleagues in Canadian children aged 2–8 years but with different age group and ancestry population [50]. Similarly, a study by Haga et al. reported that BMI trajectories varied between boys and girls in Japanese children aged between 0 and 12 years [51]. Ventura et al. [46] reported four distinct BMI trajectories in girls only. Few studies have focused on gender differences in BMI trajectories. American, Canadian, Australian and Dutch studies in children reported either three or four-class distinct BMI

Table 2 Study characteristics by BMI trajectory in girls: The Birth to Twenty Cohort

	Normal weight ($n = 717$)	Trajectory 2 ($n = 142$)	Trajectory 3 ($n = 45$)	Trajectory 4 ($n = 43$)	P value
Study characteristics					
Birth weight (kg)	3.0 (0.5)	3.0 (0.5)	3.0 (0.5)	3.3 (0.5)	0.01*
Height at 18 years (cm)	159.7 (6.2)	158.4 (5.1)	157.3 (6.6)	159.3 (6.1)	0.03*
SBP at 18 years (mm Hg)	114.3 (9.6)	116.0 (9.5)	117.9 (11.7)	120.1 (11.3)	0.00***
DBP at 18 years (mm Hg)	71.5 (8.3)	72.8 (8.1)	75.8 (12.7)	75.8 (9.0)	0.00***
MAP at 18 years (mm Hg)	85.8 (7.9)	87.2 (7.8)	89.8 (11.8)	90.6 (8.9)	0.00***
Elevated BP n (%)	187 (28.5)	28 (31.1)	17 (43.6)	18 (46.1)	0.03*

For girls: Trajectory 2 = Late Onset Overweight, Trajectory 3 = Early Onset Obese to Overweight, Trajectory 4 = Early Onset Obese to Morbidity Obese
Summary statistics presented as mean (±standard deviation) otherwise stated
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$
ANOVA and chi square test were conducted to describe the study characteristics differences in BMI trajectories
Percentages may not add up to 100 % due to rounding

Table 3 Study characteristics by BMI trajectory in boys: the birth to twenty cohort

	Normal weight ($n = 813$)	Trajectory 2 ($n = 53$)	Trajectory 3 ($n = 11$)	P value
Study characteristics				
Birth weight (kg)	3.1 (0.5)	3.2 (0.6)	3.2 (0 .7)	0.46
Height at 18 years (cm)	170.6 (6.7)	172.4 (5.5)	170.4 (7.6)	0.21
SBP at 18 years (mm Hg)	121.0 (10.8)	124.2 (8.2)	134.5 (16.9)	0.00***
DBP at 18 years (mm Hg)	71.0 (8.6)	71.9 (8.3)	76.8 (8.7)	0.09
MAP at 18 years (mm Hg)	87.7 (8.1)	89.4 (7.3)	96.0 (10.3)	0.00**
Elevated BP n (%)	276 (38.7)	20 (46.5)	6 (60.0)	0.24

For boys: Trajectory 2 = Early Onset Overweight to Normal Weight, Trajectory 3 = Early Onset Overweight to Obese
Summary statistics presented as mean (±standard deviation) otherwise stated
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$
ANOVA and chi square test were conducted to describe the study characteristics differences in BMI trajectories
Percentages may not add up to 100 % due to rounding

trajectories in both girls and boys [29, 41, 45, 52–57]. Individuals in trajectories characterized by high BMI trends had an increased risk of developing hypertension in late adolescence compared to those in a normal weight trajectory in the current study.

Prevalence of elevated BP in late adolescence was relatively higher in boys (39.43 %) than in girls (30.38 %). This prevalence is consistent with a recent study in urban South African children that reported the prevalence of prehypertension or hypertension ranging between 17.6 and 40.8 % [12]. Another study in youth aged 15 to 24 years in rural Ghana reported levels of prehypertension or hypertension with a prevalence of 37.4 %. The prevalence of prehypertension or hypertension (48.8 vs. 26.6 %) was higher in males than in females [17]. It should be pointed out that the prevalence in the current study was also higher than that previously reported in some studies within and outside South Africa. A study done by Makgae and colleagues in rural South African children aged 6 to 13 years found that 1.0 to 5.8 % of boys and 3.1 to 11.4 % of girls were hypertensive [58]. Moselakgomo et al. assessed BMI and blood pressure among adolescent school children aged 10 to16 years in Limpopo province, South Africa and the results revealed that prevalence of hypertension was between 2.3 and 5.9 % [59]. The following blood pressure prevalence has been reported in other African countries. In Brazzaville, Congo 24.3 % in girls and 16.6 % prevalence of hypertension in boys school children aged 5 to 18 years [60]. In Egyptian adolescents aged 11–19 years revealed that the prevalence rates of prehypertension and hypertension were 5.7 and 4.0 %, respectively [16]. The mechanisms of the observed sex differences in elevated BP in our study sample are not clear but may be attributed to sexual dimorphism as observed in other BP control programs [61] and also due to cardiovascular fetal programming as reported before [61, 62].

Girls in the early onset obesity trajectories were likely to have elevated SBP, DBP and MAP in late adolescence compared to those in a healthy trajectory. They displayed a 118 % increased odds of developing hypertension in late adolescence compared to those in the normal weight trajectory. Boys in early onset overweight to obese trajectory were likely to have a higher SBP, DBP and MAP in late adolescence. For instance boys in this trajectory had a 13 mm Hg mean SBP higher than those in a normal weight trajectory.

One longitudinal study on life-course adiposity and blood pressure in late adolescents has been done in Australian children [63], three of the seven adiposity trajectories were associated with elevated blood pressure at 17 years old of age. We report that being in an early onset obese or overweight trajectory was associated with increased risk of elevated BP in both girls and boys which is in agreement with other studies, although comparative studies were performed in a different population and different age range [63]. Girls in the early onset obesity to overweight BMI trajectory had reduced risk of elevated BP in late adolescence compared to those who were in early onset obesity to morbidity obese trajectory despite the fact that both groups had early onset obesity. This study was not an intervention study but it is important to study the mechanisms behind the weight loss in this group of girls. This could be important in reducing hypertension risk later in life.

The current study has several strengths; firstly, the longitudinal study in black South African children is a representative sample of South African children hence more relevant in understanding the different BMI trajectories in black South African children. Using LCGMM in identifying trajectories is an ideal analytical method since it involves a number of criteria in selecting the best fitting model and it predicts individual class membership from the available data and its ability to deal with missing at random data. The current results therefore clearly show individual heterogeneity in BMI development in boys and girls from five years to late adolescence. Our analysis was stratified according to sex and performed in a South

Table 4 The association of BMI trajectories, birth weight and height and blood pressure at 18 years for girls

	Unadjusted	Adjusted
SBP (mm Hg)		
BMI Trajectory		
Normal Weight	Reference	Reference
Late Onset Overweight	1.77 (−0.38 to 3.93)[a]	2.07 (−0.08 to 4.21)
Early Onset Obese to Overweight	3.66 (0.50 to 6.82)*	4.18 (1.03 to 7.33)**
Early Onset Obese to Morbidity Obese	5.87 (2.71 to 9.03)**	6.08 (2.93 to 9.23)**
Birth Weight (kg)		−0.61 (−1.99 to 0.78)
Height (cm) at 18 years		0.22 (0.11 to 0.34)**
R^2	0.02	0.04
DBP (mm Hg)		
BMI Trajectory		
Normal Weight	Reference	Reference
Late Onset Overweight	1.33 (−0.57 to 3.22)	1.40 (−0.50 to 3.30)
Early Onset Obese to Overweight	4.29 (1.52 to 7.07)**	4.44 (1.65 to 7.22)**
Early Onset Obese to Morbidity Obese	4.25 (1.48 to 7.03)**	4.28 (1.50 to 7.07)**
Birth Weight (kg)		−0.05 (−1.27 to 1.18)
Height (cm) at 18 years		0.06 (−0.04 to 0.16)
R^2	0.02	0.02
MAP (mm Hg)		
BMI Trajectory		
Normal Weight	Reference	Reference
Late Onset Overweight	1.47 (−0.33 to 3.28)[a]	1.62 (−0.19 to 3.43)
Early Onset Obese to Overweight	4.08 (1.43 to 6.73)***	4.35 (1.70 to 7.01)***
Early Onset Obese to Morbidity Obese	4.79 (2.14 to 7.44)***	4.88 (2.23 to 7.54)***
Birth Weight (kg)		−0.23 (−1.40 to 0.93)
Height (cm) at 18 years		0.12 (0.02 to 0.21)*
R^2	0.03	0.03
Elevated BP at 18 years (Normal BP-reference)	OR	OR
BMI Trajectory		
Normal Weight	Reference	Reference
Late Onset Overweight	1.13 (0.70 to 1.82)	1.14 (0.71 to 1.84)
Early Onset Obese to Overweight	1.93 (1.00 to 3.72)*	1.95 (1.01 to 3.77)*
Early Onset Obese to Morbidity Obese	2.15 (1.11 to 4.12)*	2.18 (1.31 to 4.20)*
Birth Weight (kg)		0.93 (0.67 to 1.27)
Height (cm) at 18 years		1.01 (0.98 to 1.03)
Pseudo R^2	0.01	0.01

SBP systolic blood pressure, *DBP* diastolic blood pressure, *MAP* mean arterial pressure, *OR* odds ratio
Adjusted for birth weight and height at 18 years
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$
[a]Intercept (MAP), OR (elevated BP) and 95 % Confidence Interval are presented

Table 5 The association of BMI trajectories, birth weight and height and blood pressure at 18 years for boys

	Unadjusted	Adjusted
SBP (mm Hg)		
BMI Trajectory		
Normal Weight	Reference	Reference
Early Onset Overweight to Normal Weight	3.23 (−0.08 to 6.54)[a]	2.85 (−0.43 to 6.13)
Early Onset Overweight to Obese	13.46 (6.75 to 20.17)**	13.45 (6.82 to 20.07)**
Birth Weight (kg)		−1.35 (−2.85 to 0.14)
Height (cm) at 18 years		0.27 (0.15 to 0.38)**
R^2	0.02	0.05
DBP (mm Hg)		
BMI Trajectory		
Normal Weight	Reference	Reference
Early Onset Overweight to Normal Weight	0.95 (−1.69 to 3.59)	0.844 (−1.80 to 3.49)
Early Onset Overweight to Obese	5.77 (0.41 to 11.13)	5.74 (0.39 to 11.10)*
Birth Weight (kg)		−0.53 (−1.7 to 0.68)
Height (cm) at 18 years		0.07 (−0.2 to 0.17)
R^2	0.01	0.01
MAP (mm Hg)		
BMI Trajectory		
Normal Weight	Reference	Reference
Early Onset Overweight to Normal Weight	1.71 (−0.77 to 4.19)[a]	1.51 (−0.96 to 3.99)
Early Onset Overweight to Obese	8.33 (3.3 to 13.37)***	8.31 (3.30 to 13.32)***
Birth Weight (kg)		−0.80 (−1.93 to 0.33)
Height (cm) at 18 years		0.14 (0.05 to 0.22)**
R^2	0.02	0.03
Elevated BP at 18 years (Normal BP-reference)	OR	OR
BMI Trajectory		
Normal Weight	Reference	Reference
Early Onset Overweight to Normal Weight	1.38 (0.74 to 2.55)	1.34(0.72 to 2.50)
Early Onset Overweight to Obese	2.38 (0.66 to 8.49)	2.39 (0.67 to 8.57)
Birth Weight (kg)		0.89 (0.66 to 1.18)
Height (cm) at 18 years		1.02 (1.00 to 1.05)*
Pseudo R^2	0.003	0.01

SBP systolic blood pressure, *DBP* diastolic blood pressure, *MAP* mean arterial pressure; *OR* odds ratio
Adjusted for birth weight and height at 18 years
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$
[a]Intercept (MAP), OR (elevated BP) and 95 % Confidence Interval are presented

African black population, which gives us an in-depth understanding of the difference in BMI developmental patterns in black boys and girls in South Africa. These

results suggest that targeted intervention might be developed for individuals in high-risk trajectories. Limitations of our current study include the fact that the sample used in this study is from Soweto, South Africa and generalizability to other parts of Africa should be treated with caution. Adiposity trajectories explain only 5 % variation in late adolescent blood pressure and we do not know yet what the other factors are, that would explain the remaining variance. We speculate that the role of genetics and epigenetics could be important, as previously reported [64]. The role of genetics and epigenetics on adiposity and growth patterns in African populations is understudied and it calls for further investigation. A recent study by Kagura and colleagues in this cohort reported no association between blood pressure at 18 and alcohol consumption or smoking or both at 18 years in this population [65] and thus we did not include these variables within the models. Furthermore, we examined walking to school as a proxy of physical activity but this variable was not able to different among participants given that most participants are walking extensively during the day to and from school.

Due to the limited sample size we might not be able to capture all trajectories; this also influenced the observation that the high-risk trajectory in boys comprised of very few individuals that might influence association analysis.

Conclusions

In this study, we identified distinct sex-specific trajectories. The early onset obesity or overweight trajectories are associated with elevated blood pressure in late adolescence. These results signify the importance to consider patterns of BMI development, especially at early stage of development, so that prevention strategies may be implemented to target those individuals in high-risk developmental patterns.

Our study has shown that patterns of adiposity could be a preferred predictor of future SBP, DBP, MAP and elevated BP in late adolescents compared to cross-sectional BMI measures. Furthermore, identification of childhood obesity can help with early identification of those at risk of developing elevated BP and other chronic diseases in adulthood.

Abbreviations

BMI, body mass index; DBP, diastolic blood pressure; LCGA: latent class growth analysis; LCGMM: latent class growth mixture modeling; MAP, mean arterial pressure; SBP, systolic blood pressure

Acknowledgments

We wish to thank the Bt20 participants for taking part in the study and the Bt20 team for their relentless support throughout the study.

Funding

The Bt20 is supported by the Wellcome Trust (UK) [grant number 092097/Z/10/Z], the South African Medical Research Council, the University of Witwatersrand and DST-NRF Centre of Excellence in Human Development, University of the Witwatersrand, Johannesburg, South Africa funding for the collection, cleaning and storage and management of the data. This work is based on the research supported in part by the Thuthuka Programme of the National Research Foundation of South Africa [Award No. 94007]. The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF. Norris is supported by the UK MRC/DFID African Research Leader Scheme.

Availability of data and materials

Data on this study is available on request from the Developmental Pathways for Health Research Unit data management department by contacting Prof. Shane A Norris.

Authors' contributions

RM, JK, ZL and SAN carried out the initial conceptualization and design of the study. RM led the study, conducted the initial analyses and interpreted the data, drafted the initial manuscript, and approved the final manuscript as submitted in close collaboration with JK, ZL and SAN who gave guidance throughout the study from conception to the approval of the final draft, reviewed and edited the data analysis plan and the manuscript for intellectual content. All authors read and approved the final manuscript as submitted.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Prior to the study, ethical approval was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg (Reference number M0101556), participants or their parents/caregivers when the participants were minor gave informed consent at the beginning of each data collection session throughout the study.

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Received: 21 April 2016 Accepted: 21 July 2016
Published online: 29 July 2016

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CHILDHOOD OBESITY
Month 2017 | Volume X, Number X
© Mary Ann Liebert, Inc.
DOI: 10.1089/chi.2016.0310

ORIGINAL ARTICLE

Early Life Growth Predictors of Childhood Adiposity Trajectories and Future Risk for Obesity: Birth to Twenty Cohort

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Abstract

Background: There is growing evidence of variations in adiposity trajectories among individuals, but the influence of early life growth patterns on these trajectories is underresearched in low- and middle-income countries. Therefore, our aim was to examine the association between early life conditional weight gain and childhood adiposity trajectories.

Methods: We previously identified distinct adiposity trajectories (four for girls and three for boys) in black South African children (boys = 877; girls = 947). The association between the trajectories and early life growth patterns, and future obesity risk was assessed by multivariate linear and multinomial logistic and logistic regressions. Conditional weight gain independent of height was computed for infancy (0–2 years) and early childhood (2–4 years).

Results: Conditional weight gain before 5 years of age was significantly associated with early onset of obesity or overweight (excess weight) BMI trajectories in both boys and girls. In girls, greater conditional weight gain in infancy was associated with increased relative risk of being in the early-onset obese to morbid obese trajectory, with relative risk ratios of 2.03 (95% confidence interval: 1.17–3.52) compared to belonging to a BMI trajectory in the normal range. Boys and girls in the early-onset obesity or overweight BMI trajectories were more likely to be overweight or obese in early adulthood.

Conclusions: Excessive weight gain in infancy and early childhood, independent of linear growth, predicts childhood and adolescent BMI trajectories toward obesity. These results underscore the importance of early life factors in the development of obesity and other NCDs in later life.

Keywords: BMI trajectories; childhood obesity; latent classes; latent class growth mixture modeling; weight gain

Introduction

Obesity has become a worldwide growing public health burden.[1–5] Excess adiposity is associated with greater risk for chronic diseases such as type 2 diabetes mellitus, hypertension, and several types of cancer and increased mortality.[6–11] Understanding obesity trajectories requires longitudinal research that would accurately recognize individuals at higher risk of becoming overweight or obese, and more importantly, identify critical periods of intervention.

There is growing evidence that studying weight gain across the life-course is essential in identifying early life determinants and future risk of obesity.[12–17] However, the majority of longitudinal studies related to weight gain and obesity from childhood to adulthood are from high-income countries, with a paucity of research focused on low- or middle-income countries, where obesity rates are on the rise. Birth weight, both low and high, has widely been used as an indicator of future risk of obesity and different noncommunicable diseases such as type 2 diabetes and stroke, among others.[18–20] Since birth weight is a proxy for

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intrauterine growth,[21] there is a need to understand the role of other early life factors, such as postnatal weight gain, on future risk of undesirable health outcomes.

Conditional growth measures, such as conditional weight and conditional height, have widely been employed to measure uncorrelated growth rate between repeated growth measures for the past decade.[22–25] Conditional weight shows a deviation in weight of an individual from the expected size on the basis of their own previous measures and the growth of the other children in the study population.[26] Using early life conditional weight, we eliminate the influence of early length/height gain in predicting the future risk of obesity, since height gain has been reported to be protective to obesity.[27] Both conditional weight and conditional height at different growth periods have been reported to be associated with different cross-sectional body composition measures later in life,[21,22,26] but the influence of early life relative weight in infancy and early childhood on life-course BMI measures is not well established.

Data from the urban Birth to Twenty (Bt20) cohort, Africa's longest running birth cohort study, reported that being overweight or obese in both infancy and early childhood was strongly associated with obesity in late adolescence.[28]

To better understand the development of obesity in South African children, we previously employed modeling techniques that can identify BMI trajectories across childhood (age 5 years) to late adolescence (age 18 years). Through this technique, we identified four adiposity trajectories for girls and three for boys in the Bt20 cohort.[29] These results underpin the existence of variation in childhood BMI development patterns, which were observed from as early as 5 years of age. It is therefore important to understand which early life factors may accelerate an individual's progression from a normal BMI trajectory pattern to one characterized with an elevated BMI.

We hypothesized that early life growth factors such as birth weight and excessive postnatal weight gain are associated with adiposity trajectories from childhood to late adolescence. Therefore, in this study, we endeavored to establish if early life conditional weight gain was associated with membership of specific BMI growth trajectories. Furthermore, we investigated if affiliation with a specific BMI trajectory would predict an increased risk of being excess weight in young adulthood at the age of 23 years.

Materials and Methods

Study Sample

We analyzed data from the Bt20, a birth cohort of predominantly black South African (78.4%) children ($n = 3273$ at the time of enrolment), born in Soweto, Johannesburg, South Africa. The detailed cohort profile with recruitment and cohort attrition details has been described elsewhere.[30] Only those participants with BMI trajectory data were included in this study. Data on demographic and socioeconomic variables were collected at birth or soon thereafter.[31] Ethics approval for this work was obtained from the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg (Reference number M0101556); participants or their caregivers provided written informed consent throughout the study.

Assessment of Growth

Birth weight, weight, and height at subsequent data collecting dates were collected by trained research assistants. Weight was measured using a digital scale to the nearest 0.1 kg with participants in light clothes and without shoes. A wall-mounted stadiometer (Holtain, United Kingdom) was used to measure standing height to the nearest 0.1 cm. Weight and height at 5 years and 7–18 years were used to calculate BMI (weight [kg]/height [m^2]) at corresponding years.

We dichotomized BMI in early adulthood as normal (BMI <25.0 kgm^{-2}) and overweight (BMI ≥25.0 kgm^{-2}) using the Extended international (International Obesity Task Force; IOTF) BMI cutoffs.[32] Due to a limited number of individuals being categorized as obese (BMI ≥30 kgm^{-2}), we included those characterized as overweight and obesity as one group and defined it as excess weight. Birth weight and weight-for-age Z-scores were computed using the WHO growth standards.[33] Due to high correlation of repeated weight measures, conditional weight gain independent of height was calculated and used where appropriate. Conditional weight gain independent of height gain was calculated in infancy (0–2 years) and (2–4 years with average age of 5 years) as standardized residuals (SR) from sex-specific linear regressions of current weight on prior weights, heights, and current height. For instance, conditional weight gain at 2 years of age was calculated as SR from regressing weight at 2 on birth weight, birth length, and height at 2 years.[22,24] A positive conditional weight gain value indicates growing faster than expected, given prior size.

Confounders and Covariates

We selected potential confounders based on prior knowledge in published literature and possible association with the outcome variable. These include mother's height (measured soon after birth of the child), gestational age at birth (preterm = 28–36 weeks, full term >37 weeks), mother's age at delivery (categorized as <25, 25–29, and ≥30), and parity (categorized as two or less live births = 1; > two live births = 2). Socioeconomic status (SES) was calculated as a composite variable score that captures the family social class in the first 24 months of life and was represented as the sum of household assets reported by the mother or caregiver.[34] Maternal education was categorized into two categories: up to high school and beyond high school.

Childhood Adiposity Trajectories

Latent class growth mixture modeling was previously used to identify four (girls) and three (boys) distinct BMI trajectories in the same group of Bt20 participants,[29] and

were used as outcome variables in this study. Trajectory group membership was previously reported in 1824 black South African children (boys = 877, girls = 947) with at least two age-point BMI measures between 5 and 18 years of age. On average, seven BMI age-point measures were available per participant. Each individual was assigned to the class where they had a high likelihood to belong. For girls, the four reported BMI trajectory classes were defined as group 1 (''normal weight'': 75.7%), group 2 (''early-onset obesity to morbidly obese'': 4.5%), group 3 (''early-onset obesity to overweight'': 4.8%), and last, group 4 (''late-onset overweight'': 15%). For boys, three BMI trajectory classes were reported. These classes were group 1 (''normal weight'': 92.7%), group 2 (''the early-onset overweight to obese trajectory'': 1.3%), and last, group 3 (''early-onset overweight to normal'': 6%). The normal weight group was used as a reference for analysis in both boys and girls.

Statistical Analysis

We used Stata version 13[35] to examine additional descriptive characteristics of the trajectory classes. We assessed the association between class membership and other early life variables such as birth weight and conditional weight gain in infancy and early childhood, using multinomial logistic regression. Finally, we used both logistic and multivariate linear regressions to estimate the relationship between BMI latent class membership and excess weight in early adulthood at the age of 23 years. Analysis of variance and chi-square test results were used to determine differences in various study characteristics among the BMI trajectory classes with a cutoff for significance set at 5%.

Results

We compared maternal education, parity and weight status (categorical) in each BMI trajectory class using chi-square tests and compared the means of continuous early life factors across BMI trajectories. In girls, maternal education, socioeconomic status, birth weight, maternal height, and weight status in early adulthood were significantly different at a 5% confidence level among the adiposity trajectories (Table 1). Only maternal education, weight status in early adulthood, and gestation period were

Table 1. Study Characteristics by Body Mass Index Trajectory in Girls: The Birth to Twenty Cohort

Study characteristics	Group 1 (normal weight: $n = 755$)	Group 2 (early-onset obese to morbidity obese: $n = 42$)	Group 3 (early-onset obese to overweight: $n = 43$)	Group 4 (late-onset overweight: $n = 107$)	p
Maternal education, *n* (%)[a]					
Up to secondary school	652 (86.4)	39 (92.9)	31 (72.1)	96 (89.7)	0.02*
≥High school	103 (13.6)	3 (7.1)	12 (27.9)	11 (10.3)	
Parity/birth order, *n* (%)					
≤2 children	521 (69.0)	32 (76.2)	30 (69.8)	71 (66.4)	0.71
>2 children	234 (31.0)	10 (23.8)	13 (30.2)	36 (33.6)	
SES score	4.85 (2.05)	5.03 (1.79)	5.58 (1.50)	4.34 (1.92)	0.03*
Birth weight (kg)	3.0 (0.5)	3.3 (0.5)	3.0 (0.5)	3.0 (0.5)	0.01*
Birth weight (z score)	–0.55 (1.17)	0.01 (1.18)	–0.67 (1.09)	–0.53(1.13)	0.01*
Maternal height (cm)	158.56 (5.82)	160.67 (5.72)	158.13 (5.60)	159.04 (5.13)	0.04*
Maternal age (years)	25.6 (6.2)	26.3 (5.3)	26.9 (6.7)	25.5 (6.4)	0.47
Gestational age (weeks)	37.8 (1.9)	38.3 (2.0)	38.2 (2.0)	37.8 (1.6)	0.54
Weight status at 23 years, *n* (%)					
Normal weight, *n* (%)	299 (62.2)	1 (3.1)	5 (19.2)	20 (28.6)	0.000***
Overweight, *n* (%)	135 (28.1)	3 (9.4)	7 (26.9)	27 (38.6)	0.000***
Obese, *n* (%)	47 (9.7)	28 (87.5)	14 (53.9)	23 (32.8)	

Summary statistics presented as mean (±standard deviation) otherwise stated.

*$p < 0.05$; ***$p < 0.001$.

ANOVA and chi-square test were conducted to describe the study characteristics differences in BMI trajectories.

[a]Percentages may not add up to 100% due to rounding.

ANOVA, analysis of variance; SES, socioeconomic status.

Table 2. Study Characteristics by Body Mass Index Trajectory in Men: The Birth to Twenty Cohort

Study characteristics	Group 1 (normal weight: $n = 816$)	Group 2 (early-onset overweight to obese: $n = 11$)	Group 3 (early-onset overweight to normal weight: $n = 50$)	p
Maternal education, *n* (%)[a]				
Up to secondary school	697 (85.4)	7 (63.6)	38 (76.0)	0.03*
≥High school	119 (14.6)	4 (36.4)	12 (24.0)	
Parity/birth order, *n* (%)				
≤2 children	540 (66.2)	7 (63.6)	36 (72.0)	0.69
>2 children	276 (33.8)	4 (36.4)	14 (28.0)	
SES score	4.80 (2.07)	6.29 (1.70)	5.34 (1.98)	0.06
Birth weight (kg)	3.1 (0.5)	3.2 (0 .7)	3.2 (0.6)	0.46
Birth weight (z score)	–0.52 (1.10)	–0.45 (1.64)	–0.34 (1.17)	0.52
Maternal height (cm)	158.5 (6.00)	160.6 (8.0)	158.7 (5.6)	0.99
Maternal age (years)	25.8 (6.3)	26.2 (6.1)	25.3 (6.0)	0.99
Gestational age (weeks)	37.9 (1.8)	38.7 (2.3)	38.4 (1.4)	0.00***
Weight status at 23 years, *n* (%)				
Normal weight	496 (87.0)	2 (28.6)	11 (44.0)	0.000***
Overweight	66 (11.6)	1 (14.3)	11 (44.0)	
Obese	8 (1.4)	4 (57.1)	3 (12.0)	

Summary statistics presented as mean (± standard deviation) otherwise stated.

*$p < 0.05$; ***$p < 0.001$.

ANOVA and chi-square test were conducted to describe the study characteristics differences in BMI trajectories.

[a]Percentages may not add up to 100% due to rounding.

significantly different in boys (Table 2). This means that the education level varied across the BMI trajectories with those who did not go beyond high school likely to be assigned in the early-onset excess weight trajectories in both young adult men and women. For weight status, as expected, those with excess weight in early adulthood were likely to belong to the early-onset excess weight trajectory membership.

The average BMI values from 5 to 18 years are presented in Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/chi). Average BMI for boys (20.4 kg/m^2) was lower compared with girls (23.3 kg/m^2) at 18 years. The life-course BMI-for-age prevalence for overweight from childhood (at 5 years) to late adolescence (at 18 years) using IOFT cut offs showed a steady increase in prevalence in girls than in boys. At 5 years of age, the prevalence of excess weight was 11.06% for boys and 13.58% for girls. At 9 years of age, the prevalence of excess weight for girls was twice higher than in boys, 15.3% and 8.8%, respectively. This trend continued up to late adolescence, where at 18 years of age, prevalence of excess weight was thrice more in girls (33.8%) than boys (9.6%).

Predictors of Membership within Each Trajectory

Compared with those in normal range weight trajectory, conditional weight gain in infancy (relative risk ratios [RRR] = 2.0, 95% confidence interval [CI]: 1.2–3.5) was significantly associated with the early-onset obesity to morbid obesity trajectory in girls. In addition, conditional weight gain in early childhood was significantly associated (RRR = 1.8, 95% CI: 1.09–3.0) with the early-onset obesity to overweight trajectory (Table 3). Similarly, in boys, conditional weight gain independent of linear growth in infancy is significantly associated with early-onset overweight to normal weight (RRR = 2.5, 95% CI: 1.52–4.1) and early-onset overweight to obesity (RRR = 5.4, $p < 0.01$) trajectories, compared to the normal weight (Table 3). Birth weight and maternal height were not associated with any of the BMI trajectory classes in both girls and boys.

The prevalence of excess weight in early adulthood at the age of 23 years was 46.6% in women and 15.5% in men. Women in the early-onset obese to morbidly obese trajectory had a 44.8-fold increased risk of excess weight compared to those in normal weight trajectory, while men in early-onset overweight to obese trajectory had a 13.6-fold increased risk (Supplementary Table S2).

Table 3. Relative Risk Ratios and 95% Confidence Intervals of the Predictors of BMI Trajectory Membership Compared with the "Normal" Group for Girls and Boys

Trajectories (Girls)	Unadjusted	Adjusted
Normal weight	—	—
Early-onset obese to morbidity obese	—	—
Birth weight (kg)	2.61 (1.03–6.57)*	1.30 (0.41–4.06)
Relative weight gain 0–2 years (z-score)	1.96 (1.29–3.45)**	2.03 (1.17–3.52)*
Relative weight gain 2–4 years (z-score)	1.18 (0.76–1.82)	0.99 (0.61–1.59)
Mother height (cm)	1.07 (0.98–1.17)	1.06 (0.96–1.17)
Pseudo R^2	0.04	0.06
Early-onset obese to overweight	—	—
Birth weight (kg)	1.17 (0.41–3.31)	0.74 (0.23–2.43)
Relative weight gain 0–2 years (z-score)	1.65 (0.94–2.89)	1.75 (0.97–3.17)
Relative weight gain 2–4 years (z-score)	1.51(0.92–2.46)	1.82 (1.09–3.04)*
Mother height (cm)	1.03 (0.93–1.13)	1.03 (0.93–1.14)
Pseudo R^2	0.04	0.06
Late-onset overweight	—	—
Birth weight (kg)	0.95 (0.55–1.63)	0.99 (0.52–1.87)
Relative weight gain 0–2 years (z-score)	1.04 (0.77–1.41)	1.02 (0.75–1.39)
Relative weight gain 2–4 years (z-score)	1.26 (0.97–1.63)	1.17 (0.90–1.54)
Mother height (cm)	1.03 (0.99–1.08)	1.03 (0.98–1.08)
Pseudo R^2	0.04	0.06
Trajectories (Boys)	**Unadjusted**	**Adjusted**
Normal weight	—	—
Early-onset overweight to obese		
Birth weight (kg)	0.36 (0.07–1.98)	0.52 (0.07–3.67)
Relative weight gain 0–2 years (z-score)	2.43 (1.03–5.72)*	5.40 (1.42–20.45)**
Relative weight gain 2–4 years (z-score)	0.53 (0.27–1.03)	0.51 (0.21–1.25)
Mother height (cm)	0.94 (0.75–1.17)	0.94 (0.75–1.17)
Pseudo R^2	0.14	0.24
Early-onset overweight to normal weight		
Birth weight (kg)	2.00 (0.84–4.72)	2.70 (0.93–7.88)
Relative weight gain 0–2 years (z-score)	2.39 (1.52–3.74)***	2.49 (1.52–4.08)***
Relative weight gain 2–4 years (z-score)	1.97 (1.23–3.17)**	2.27 (1.31–3.95)**
Mother height (cm)	1.00 (0.93–1.08)	1.01 (0.93–1.10)
Pseudo R^2	0.14	0.24

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Unadjusted = birth weight + relative weight gain 0–2 years + relative weight gain 2–4 years + mother height.

Adjusted = unadjusted + maternal education + social economic status + mother age + gestation age.

Discussion

We have shown that conditional weight gain independent of height in infancy and early childhood was significantly associated with different BMI trajectories, more especially early-onset obesity/overweight trajectories. For instance, a one standard deviation increase in z-score for conditional weight gain independent of linear growth between 0 and 2 years in girls resulted in an increased risk of 103% following an early-onset obesity to morbid obesity trajectory compared to those with a normal weight. The results show that excessive weight gain at any point during early childhood (before 5 years) almost exclusively determined that an individual is likely to be overweight or obese in early adulthood. This is consistent with a Brazilian study, which reported that the amount of weight gain before 5 years of age was one of the most important predictors of overweight later in life.[36] Using more detailed longitudinal data, our findings are in contrast to the results reported from five cohorts from low- and middle-income countries, including South Africa, that faster weight gain in infancy and early childhood did not pose higher risk of body composition outcomes in adulthood. Unlike results from other studies, birth weight and maternal height did not influence the subsequent BMI trajectory membership of cohort participants.[37] Our results suggest the need for taking a life-course approach to understand how early life weight affects later life health outcomes, as it provides a better understanding of trajectories and health risk outcomes.

We reported a higher prevalence of overweight and obesity in girls than in boys across childhood and in young adulthood. Women were thrice more likely to have excess weight than men in early adulthood; this is consistent with reported results on sex differences in obesity prevalence or incidence being higher in girls and more than in boys and men in South Africa.[4,28] The absolute prevalence of excess weight at 18 years of age was 33.8% in girls and 9.6% in boys, and it was 46.6% in young women and 15.5% in young men at 23 years of age. These results are consistent with other studies in South African children, with girls having a higher prevalence of excess weight than boys,[28,38–41] but higher than those reported in most sub-Saharan Africa (SSA) countries.[42] The sex differences in prevalence of overweight or obesity are reported across other SSA countries too.[42–45] The prevalence rate of being obese or overweight (excess weight) in girls at 18 years in this study is comparable to the rates in American children aged between 6 and 19, where a third of the children are obese or overweight.[46,47] This also suggests that girls in this study are at a higher risk of transitioning into an obese trajectory, as they get older compared to boys. Individuals in trajectories characterized by high BMI values were at higher risk of being excess weight in early adulthood compared to those in a normal weight trajectory.

Since these trajectories represent early-onset obesity or overweight, previous studies have suggested that genetic and early life factors might be the driving force of these trajectories.[14,48] We confirmed that conditional weight gain independent of height between birth to 5 years influenced trajectory membership. Most of the reported factors that influence BMI trajectory membership, in American, Brazilian, and European children, have been related to maternal factors such as maternal BMI, maternal gestational weight gain, and maternal smoking during pregnancy, and family social economic status.[48–52] Our results show that child's postnatal weight gain is also important in identifying those individual in-high BMI trajectory groups, and hence an important factor to reduce future health risks. We speculate that conditional weight gain would have a similar important role in variation of BMI trajectory patterns in other populations, more especially in other black African populations.

The results also revealed that children belonging to early-onset obese or overweight were likely to have an undesirable higher measure of overweight or obesity in young adulthood. These results support the importance to consider the characteristics and risk factors associated with individual BMI trajectories, more especially at an early stage of development. It also illustrates the strong tracking of weight gain which has been reported to be associated with multiple health risks, such as childhood and adulthood obesity, blood pressure, and metabolic syndrome.[29,36,49,53,54]

This study has several strengths, the inclusion of detailed longitudinal data, which included early life factors, being first and foremost. This study is one of only a few that focuses on an African cohort, which provides a unique, in-depth understanding of how early life factors are linked to difference in BMI developmental patterns in South Africa. However, the early life factors investigated in this study explained only a small amount of variance in childhood BMI trajectories (< 25%), and it is important to consider other environmental and genetic aspects in future studies. We speculate that the role of genetic ancestry, as well as changes in physical activities and dietary patterns in the past two decades, may play a major role in determining trajectory membership.

Maternal factors that could have influenced the outcomes in this study, such as prepregnancy weight or BMI, smoking, and alcohol consumption before and during pregnancy, were not available in this study, and are a noted limitation. Other early life factors such as breastfeeding, dietary patterns data were also not available. Maternal weight on delivery was not collected, since it is influenced by other factors during pregnancy such as gestational weight gain. It should be noted that at this stage it is not possible to determine whether the early life growth risk factors assessed in this study are causally involved in obesity development, which highlights the importance of further investigation to fully elucidate causality through techniques such as Mendelian randomization.[55] Due to the small sample size for boys in the early-onset overweight trajectory (1.3%), the association results for this particular

trajectory membership could be affected, such as wider CIs resulting into tolerating false-positive estimates, and subsequently should be applied or inferred to other South African children with caution. These results can be generalized to other South African urban children setting, but not for rural setting where the prevalence of obesity or overweight is relatively lower compared to the urban setting.[41] Generalizability of our results to other African populations should be treated with caution since the sample for this study is from an urban South African population setting.

Conclusions

Regardless of the period of rapid weight gain, conditional weight increased the risk of an individual to belong to a BMI trajectory characterized with a consistently elevated BMI. Consequently, targeting appropriate weight gain in preschool years might lower the risk of developing obesity and related metabolic disease risk in later life.

Acknowledgments

We wish to thank the Bt20 participants for taking part in the study and the Bt20 team for their relentless support throughout the study.

The Bt20 is supported by the Wellcome Trust (United Kingdom) (grant number 092097/Z/10/Z), the South African Medical Research Council, University of the Witwatersrand and DST-NRF Centre of Excellence in Human Development, University of the Witwatersrand, Johannesburg, South Africa, funding for the collection, cleaning and storage, and management of data. This work is based on the research supported, in part, by the Thuthuka Programme of the National Research Foundation of South Africa (Award No. 94007). The financial assistance of the National Research Foundation (NRF) toward this research is hereby acknowledged. Opinions expressed and conclusions arrived at are those of the author and are not necessarily to be attributed to the NRF. Norris is supported by the UK MRC/DFID African Research Leader Scheme.

Author Disclosure Statement

No competing financial interests exist.

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