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ASSOCIATIONS OF EARLY LIFE GROWTH WITH HEALTH AT AGE 22 YEARS AS MEASURED BY AN ALLOSTATIC LOAD INDEX: BIRTH TO TWENTY PLUS COHORT

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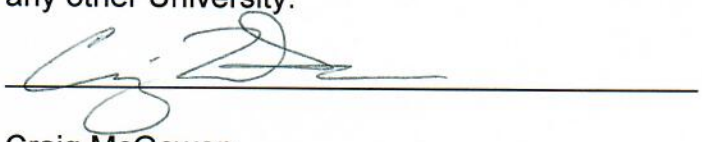
A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science.

Supervisor: Professor Shane Norris

Johannesburg, 2019

Declaration:

I, Craig McGowan, declare that this Dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



Craig McGowan

5 day of September, 2019 in Washington, DC, USA

Publications

McGowan CJ and Norris SA. Associations of early life growth with health using an allostatic load score in young, urban African adults: Birth to Twenty Plus Cohort. Journal of Developmental Origins of Health and Disease. Submitted 7 March 2019

Abstract

Growth in early life is associated with various individual health outcomes in adulthood, but limited research has been done on associations with a more comprehensive measure of health. Combining information from multiple biological systems, the allostatic load score provides such a quantitative measure of overall physiological health. Using longitudinal data from the Birth to Twenty Plus cohort in South Africa, an allostatic load score in young adulthood (at age 22 y) was calculated and associations with birth weight and linear growth and weight gain from age 0-2 y and 2-5 y were examined, as mediated by trajectories of body mass index and pubertal development in later childhood and adolescence. Missing measures of components of the allostatic load measure were addressed using multiple imputation by chained equations. Association with total allostatic load score were assessed using Poisson regression, while associations with high allostatic load was assessed using logistic regression. Differences in total allostatic load score between males and females were small, though levels of individual biological factors contributing to allostatic load differed by sex. Increased weight gain from age 2 to 5 y among males was associated with an increased risk of high allostatic load, but no other early life measures were associated with allostatic load. Increased adiposity through childhood and adolescence in females was associated with higher allostatic load in early adulthood. These results illustrate that patterns of early life growth are not consistently associated with a higher allostatic load. While more research is needed to link allostatic load in young adulthood to later health outcomes in settings like South Africa, these results suggest that increased adiposity during childhood and adolescence represents a critical factor and potential early sign of later physiological health risk.

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Nomenclature

AL – allostatic load

BMI – body mass index

Bt20+ - Birth to Twenty Plus

CHD – coronary heart disease

CRP – C-reactive protein

DBP – diastolic blood pressure

DOHaD – developmental origins of health and disease

GHQ-28 – 28 item General Health Questionnaire

HDL – high density lipoprotein

MS – metabolic syndrome

NHANES – National Health and Nutrition Examination Survey

SBP – systolic blood pressure

SES – socio-economic status

WHR – waist-hip ratio

CHAPTER 1: LITERATURE REVIEW

1.1 Overview:

Events and experiences in an early stage of the life course are increasingly recognised as having the potential for impacts on health outcomes much later. This concept is outlined in the 'developmental origins of health and disease' (DOHaD) framework, which outlines how preconception, faetal, and early life events can result in lasting physiological changes. Gluckman (1) summarised the development of the DOHaD framework beginning in the 1970's and 1980's with initial research linking early life conditions with cardiovascular and metabolic outcomes later in adulthood (2-5). As the field grew, theoretical models to explain the observed outcomes were developed and began to mature, positing that an individual adjusts its development based on its surrounding environment, but that these adjustments may have deleterious long-term consequences (6-9). A variety of mechanisms have been proposed to explain these observed associations across stages of the life course, including epigenetics, hormonal adaptations, and alterations to the microbiome (10).

Figure 1.1 shows a conceptual model outlining an example of the DOHaD framework, illustrating hypothesized associations of one such factor from early life, growth, and its association with adult health, mediated by growth and puberty in later childhood and adolescence and impacted by external factors such as socio-economic status (SES) and maternal factors.

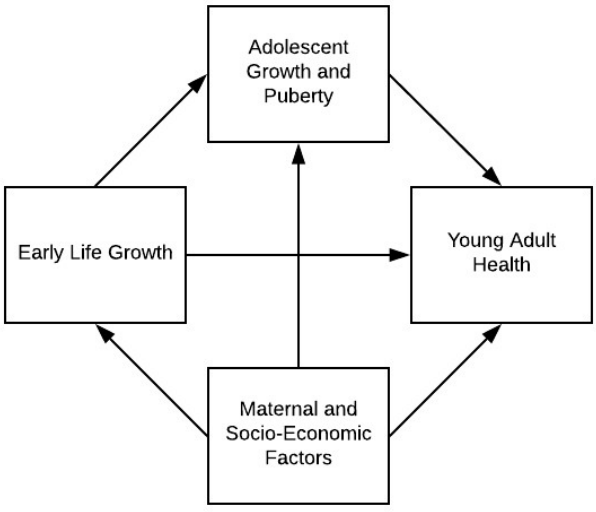


Figure 1.1. Conceptual model linking early life growth, adolescent growth, and young adult health

While there has been considerable research into associations between early life exposures and individual health outcomes later in life, less is known about links between early life events and more comprehensive measures of adult health. Once

such measure, developed contemporaneously with the DOHaD framework, is the concept of allostatic load (AL). As proposed by McEwen and Stellar (11), AL is a measure of physiological effects of exposure to chronic stressors that result in fluctuating or heightened neural and neuroendocrine responses. This physiological 'wear and tear' can in turn result in increased susceptibility to disease. By combining measures of multiple physiological systems into a single framework, a measure of AL can provide a more holistic snapshot of health than individual measures.

In this review, I begin by outlining what is known about links between early life, adolescence, and individual adult measures of health. Following that, I explore the development of measures of AL, potential influences of AL, and known associations of AL with further health outcomes. I conclude by illustrating the research gaps that exist in linking the DOHaD framework to a comprehensive AL measure.

1.2 Associations between early life factors and childhood or adolescent health

1.2.1 Adiposity

Many studies exploring associations between early life and childhood or adolescent health have focused on body composition and adiposity as outcomes. A 2005 review by Baird et al (12) found consistent evidence that both infant obesity and rapid growth in infancy were associated with higher risks of obesity later in childhood and adolescence. In 2016, Woo Baidal et al (13) reviewed 282 studies exploring risk factors for childhood obesity in the first 1000 days of life. Consistent early life risk factors identified in that review included birth weight and high infant weight gain, both of which were associated with later childhood overweight. For birth weight, 24 of 28 examined studies identified an association between higher birth weight and overweight, and 45 of 46 examined studies identified an association between high infant weight or weight gain and overweight. Significantly, this review included only results from prospective cohort studies with measured outcomes and did not include any studies using self-reported outcome or utilizing a cross-sectional or retrospective study design, increasing the strength of their findings.

These results are supported by similar, subsequent findings in the Birth to Twenty Plus (Bt20+) cohort in Soweto-Johannesburg, South Africa (upper middle-income country). Munthali et al (14) examined associations between birth weight and conditional growth in early childhood with identified body mass index (BMI) trajectories from age 5 y to age 18 y and weight status at age 23 y. Greater conditional weight gain prior to age 5 y was found to be associated with trajectories corresponding to early-onset overweight or obesity in both boys and girls, though birth weight was not associated with any outcome trajectory. While the lack of identified association between birth weight and childhood BMI is somewhat surprising given the consistent findings of Woo Baidal et al (13), it is possible that such an association also exists in this cohort but is being mediated through later childhood growth in this context.

1.2.2 Other health measures

Besides adiposity and body composition, several studies have investigated associations with early life factors and pubertal timing. A 2007 review by Patton and Viner (15) described secular trends in pubertal timing that occurred during the twentieth century, as the mean age of menarche dropped to between ages 12-13 in most developed countries, likely influenced by improvements in early life nutrition. Using data from Bt20+, Lundeen et al (16) illustrated links between growth prior to age 5 y and trajectories of pubertal development. For both boys and girls, greater height-for-age z scores (HAZ) and BMI-for-age z scores (BMIZ) at age 5y were associated with earlier and faster pubic hair development, with similar associations observed for earlier and faster breast development in girls.

While there are few studies looking at non adiposity- or puberty-related adolescent outcomes at these early ages, using data from the Bt20+ cohort Kagura et al (17) found associations between early life growth and systolic blood pressure (SBP) trajectories calculated from serial measurements taken from ages 5 to 18 y. In both boys and girls, greater relative weight gain in infancy was associated with membership in the highest SBP trajectory.

1.2.3 Summary

Considerable evidence exists that birth weight and growth patterns in infancy and early childhood are associated with measures of adiposity and body composition throughout childhood and adolescence. Early life growth before age 5 y has been associated with both trajectories of pubertal timing and tempo as well as trajectories of childhood/adolescent SBP in one cohort, supporting links to adult blood pressure that will be explored in the next section.

1.3 Associations between early life and/or adolescent factors and adult health

1.3.1 Adiposity

There is considerable evidence supporting the association between early life factors, especially early life growth, and adiposity into adulthood. The same 2005 review by Baird (12) that described associations of infant obesity with later childhood obesity also found consistent associations between rapid early life growth and greater risk of obesity in adulthood, which was generally defined as between 20 and 35 y of age in the studies reviewed. Associations of infant size with adult obesity were less consistent; though all associations were in the expected direction, only half reached statistical significance.

The findings summarized in that review have been supported by subsequent research. Using data from the COHORTS consortium (18), a collaboration of longitudinal cohorts in five low- and middle-income countries (LMICs) in 2013, including data from Bt20+, Adair et al (19) found a positive association between each of birthweight, faster linear growth from both age 0-2 y and 2 y to mid-childhood, and faster weight gain from both age 0-2 y and 2 y to mid-childhood with adult overweight. In 2013, Slining (20) showed that certain BMI trajectories from ages 0-2 y were predictive of adult overweight status, with participants experiencing greater weight gain early in infancy generally at higher risk of overweight than those with other infant BMI trajectories. Peneau et al (21) created BMI trajectories encompassing data from age 0-10 y and showed that those in the consistently high

trajectory or those with ascending BMI in childhood were more likely to have higher adult BMI.

While the previously reported studies illustrate consistent associations between adult BMI and early life growth, BMI is known to be an imperfect measure of adiposity as it does not distinguish between lean mass and adipose tissue (22). Two studies using data from Bt20+ have directly examined associations between early life growth and specific measures of body composition. Musa et al (23) showed that birth weight, conditional weight gain from 1-2 y, and conditional weight gain from 2-4 y were each associated with both increased fat mass and fat-free mass at age 18 y. Furthermore, conditional weight gain during both time periods in early childhood was associated with a higher ratio of fat mass to fat-free mass, indicating higher adiposity. In 2018, Prioreschi et al (24) examined similar outcomes at age 22 y and found that increased relative weight gain throughout childhood and adolescence was predictive of increased fat-free soft tissue mass, fat mass, abdominal subcutaneous adipose tissue, and abdominal visceral adipose tissue. Greater relative linear growth during childhood and adolescence, however, was only consistently predictive of fat-free soft tissue mass.

1.3.2 Cardiovascular Health

Some of the earliest research in the DOHaD field focused on links between early life factors and risks of cardiovascular disease later in life (25). Studies in the intervening years have continued to expand on and clarify these associations. In 2001, Eriksson et al (26) used data from a Swedish longitudinal cohort to illustrate that low birth weight and low ponderal index were associated with an increased risk of hospital admission or death due to coronary heart disease (CHD) in adulthood. Furthermore, low weight gain during the first year of life also increased CHD risk. Owen et al (27) conducted a 2009 meta-analysis of fifteen observation studies that explored the link between early life BMI and later CHD outcomes and showed a positive association between increased BMI in later childhood and adolescence and CHD risk, though no associations with BMI in early childhood (age 2-6 y). These results illustrate the relevance of body size throughout childhood and adolescence, not just in early life, on future risks of CHD. Beyond body size, Golub et al (28)

reviewed literature on effects of altered pubertal timing and found evidence that early adrenarche is associated with a higher risk of cardiovascular disease as adults.

In addition to CHD, many studies have examined associations between early life factors and blood pressure later in life. In a 2005 review, Lawlor and Smith (29) described consistent associations between low birthweight and obesity in childhood or adolescence with higher adult blood pressure. Beyond measures of early life growth, the authors found consistent evidence from cohort studies that poor socioeconomic status (SES) in early life was associated with increased blood pressure in adulthood, even after accounting for adult SES. In addition to the associations with adult BMI described earlier, Adair et al (19) found that both faster linear growth and faster weight gain in early childhood were associated with increased risk of elevated blood pressure in adulthood. Using Bt20+ data, Munthali et al (30) showed that trajectories of BMI through childhood and adolescence were associated with blood pressure, with individuals in the early onset obesity or overweight trajectories more likely to have elevated blood pressure at age 18 y. These finding were echoed by Sabo et al (31), who found that early height or BMI growth was positively associated with increased blood pressure in adulthood. While most of the previous studies examined blood pressure in either early adulthood or middle age, Sandboge et al (32) used data from a Finnish cohort to examine associations between childhood growth and blood pressure beyond age 60 years. In contrast to other findings, they found no association between childhood growth and blood pressure at mean age 62 y and an inverse relationship with blood pressure at mean age 66 y.

1.3.3 Metabolic Health

With the increasing burden of diabetes and other metabolic conditions throughout the world (33), research into potential early life factors influencing those conditions is of great interest. In a 2011 review, Reilly and Kelly (34) found a consistent relationship between higher BMIZ scores or overweight/obesity at age 5 y and diabetes risk in adulthood, as well as increased lifetime risks of diabetes when overweight or obese at age 18 y. Using data from the COHORTS consortium, Norris et al (35) illustrated an increased risk of adult diabetes for individuals with lower birthweight or faster growth after age 4 y. McEniry's 2013 review of twenty studies in

LMICs also found consistent associations between high waist-to-hip ratio (WHR) in childhood and adult diabetes risk (36). In the same review of effects of pubertal timing, Golub et al (28) also described associations between early adrenarche and increased risk of diabetes, with the evidence in girls stronger than evidence in boys.

1.3.4 Mental Health

Beyond physical health, there is a consensus that mental health in adulthood is influenced by earlier life periods, especially adolescence. An early study to examine this relationship was conducted by Kessler et al (37) in 2005 using a nationally-representative sample of Americans. They found that about half of the respondents met the definition for at least one diagnosable mental health condition at some point in their life and that the first occurrence of those conditions often occurred in childhood or adolescence. This was reinforced in a 2007 review that focused on data from World Health Organization World Mental Health surveys, which found that the initial onset of many mental disorders occurs during childhood or adolescence (38). While the previously described studies examined timing of mental health onset, Keenan-Miller et al (39) used a longitudinal cohort to show that later perceived physical health was associated with early adolescent depression.

Substantial evidence links the onset of puberty and its associated physical and developmental changes with adverse mental health outcomes. As part of a Lancet Series on Adolescent Health, Patton and Viner (15) explained how early puberty is associated with behavioural and emotional issues, as well as physical illness such as persistence of asthma. Golub et al's 2008 review of public health effects of pubertal timing described similar findings for the associations between early puberty and higher incidence of conduct and behavioural disorders (28). Individual studies in Finland (40) and the Netherlands (41) also found similar results, where early pubertal timing was associated with increased prevalence of mental health and behaviour issues.

1.3.5 Summary

Studies in various settings have found substantial evidence to support the associations of factors in childhood and adolescence with health in adulthood. Associations between growth in childhood before age 5 y and adult adiposity are

consistent across studies and across ages, with increased growth in early life predicting both increased BMI and specific measures of adiposity in adulthood. Associations between early life growth prior to age 5 y and cardiovascular and metabolic outcomes are generally strong, though not always as consistently observed. Pubertal timing has also been shown to influence both physical and mental health into adulthood.

1.4 Allostatic Load

1.4.1 Framework

While all health outcomes explored in the previous sections are meaningful, none gives an overall picture of physiological health. Beginning in the early 1990s, McEwen and Stellar (11) extended the concept of allostasis, where the body physiologically reacts to external stresses, over time to encompass how the body reacts to cumulative stresses, a concept they called *allostatic load* (AL). They theorized that such cumulative 'wear and tear' can predispose an individual to disease later in life, and that AL would lie along the causal path from repeated stresses to eventual disease outcomes. Such a path is mediated by through the neural and neuroendocrine systems of the body, which translate the perceived environmental stresses into physiological responses.

One of the first studies to create a measure of AL was conducted in older (age 70+ y) adults by Seeman et al in 1997 (42), where they used measurements of ten biological parameters encompassing the cardiovascular, metabolic, and neuroendocrine systems. By summarising the number of risk factors for which an individual's value was in the highest-risk quartile of observed values, they created an allostatic load index with values ranging from zero to ten. This index contained four measures of the neuroendocrine system (urinary cortisol, epinephrine, norepinephrine, and dehydroepiandrosterone sulphate), which are regarded as primary mediators under the AL framework, and six secondary outcomes (SBP, diastolic blood pressure [DBP], WHR, high-density lipoprotein [HDL], ratio of total cholesterol to HDL, and glycosylated haemoglobin). This count-based AL score was found to be associated with decreasing memory and physical performance over a

two- to three-year follow-up period, as well as increased risk of new cardiovascular disease during that period. Seeman et al (42) also compared the predictive power of the AL score to those of the individual components and found that AL score was able to better predict the examined outcomes. This study provided empirical support for McEwen and Stellar's theory (11) and laid the groundwork for future developments in the operationalisation of AL.

1.4.2 Operationalisations

As the field of allostatic load research developed in the last twenty years, there have been multiple different operationalisations employed across various studies, both in terms of the physiological measures employed and the method of calculating the score. One of the first reviews of different methodologies was conducted by Beckie in 2012 (43). Examining 58 studies conducted prior to 2012, she found that many researchers used a count-based index similar to the original operationalisation, and that the few studies that specifically compared different scoring methodologies generally found that including a wide variety of indicators was more important than the choice of metric. Many studies incorporated additional biomarkers beyond the original ten, including measures of immune function such as C-reactive protein (CRP), albumin, and interleukin-6 (44, 45), or additional cardiometabolic measures such as BMI, triglycerides, and resting heart rate (46-48). Beckie found that the choice of which indicators to include was largely driven by data availability, but that a substantial number of studies did not include any measures of the neuroendocrine primary mediators. This partially reflects the challenges in assessing neuroendocrine levels, as many vary in a circadian rhythm or require extended collection periods.

These findings were reinforced in a 2015 review focusing on AL calculations in working adults (49), and two reviews in 2017 (50, 51). All emphasized the general exclusion of neuroendocrine markers from the various AL indices, and that the variations in methodologies for calculating AL made it difficult to compare results across studies effectively. To this date, there remains no 'gold standard' operationalisation of AL. Decisions as to what biomarkers to include are largely driven by data availability, with the empirical high-risk summary index originally developed by Seeman et al (42) remaining a common methodology for creating the

AL score. Many researchers use the resulting continuous AL score for their resulting analyses (47, 52-54), though others have utilised an empirical cut point based on the distribution of AL scores to create a measure of high AL (55).

1.4.3 Exposures associated with allostatic load

While the previously described variations in operationalisation of AL score make exact comparisons across studies difficult, there are several clear trends. Lower socioeconomic status, whether measured as lower education or lower income, has been found to be associated with higher AL score in multiple studies of adults. Dowd et al (56) conducted a 2009 review examining the evidence for associations between SES and AL as well as between SES and cortisol, a measure often included as a component in AL scores. Examining studies primarily conducted in among middle-age and older adults, they found inconsistent evidence for associations between SES and cortisol but more evidence for an association between lower SES and higher AL among adults. They suggested that the associations between lower SES and higher AL was primarily through impacts on the cardiovascular and metabolic components of the AL score, rather than via the neuroendocrine components. In her 2012 review, Beckie also highlighted the inverse relationship between SES and AL score (43). Johnson et al's 2017 review (51), while critical of the wide variety of operationalisations of AL employed in the 26 studies examined, noted that all but three studies had found evidence for an inverse association between SES and AL score. A final review by Ribeiro et al (57) examined the relationship between neighbourhood socioeconomic deprivation and individual AL score. Most studies they examined found an inverse relationship between neighbourhood deprivation and AL score, extending the relationship between SES and AL beyond individual status and encompassing an individual's surrounding environment.

Much of the research on predictors of allostatic load has focused on adult populations, but some studies have examined associations of AL and measures of socioeconomic position in adolescent and early adult years. Using data from multiple rounds of the US National Health and Nutrition Examination Survey (NHANES), collected from 1999 to 2008, Rainisch et al (52) found both disparities by both race and SES in AL score among adolescents aged 12 to 19 y. Evans et al (58) used a

measure of cumulative risk in childhood, which included measures of parental presence and support, stressful life events, and SES, and showed that higher levels of cumulative risk were associated with higher AL in adolescents with a mean age of ~ 13 y.

Little research has been conducted looking at associations between early life or adolescent biological measures and allostatic load later in adulthood. In 2005 Allsworth et al (55) used earlier NHANES data, collected from 1988 to 1994, to illustrate a link between early menarche and higher AL score in women ranging from 17 to 30 y old, where those with AL scores in the top quartile had more than double the odds of reporting early menarche compared to women with lower AL scores. A 2018 study in Denmark examined associations between what they described as "biomedical" and "social" factors with AL in middle age (59). The biomedical factors examined included gestational age, maternal BMI and age, and birth weight, while social factors included parental SES, maternal marital status, and whether the child lived with parents at age 1 y. Maternal BMI and age, along with parental SES, were consistently associated with adult AL in both sexes, and birth weight was associated with adult AL in females. This was the only study identified that explicitly examined associations between early life biological factors and AL in adulthood.

1.4.4 Consequences of allostatic load

Most of the research on consequences of a high allostatic load comes from studies of older adults in high-income "Western" contexts. In the first study to operationalise AL, Seeman et al (42) found that older (age 70+ y) American adults with higher AL scores had poorer cognitive and physical outcomes three years later. These findings were reinforced in a follow-up of the same cohort seven years after the initial AL measurements, in which higher AL was found to predict increased mortality, incident cardiovascular disease, declines in physical functioning, and declines in cognitive functioning (54).

More recent studies have explicitly examined links between higher allostatic load and mortality in younger and middle-aged adults. By linking biological risk measures collected from NHANES surveys to later mortality records, Crimmins et al

(48) showed that life expectancy at age 20 was lower for those individuals with increased numbers of biological risk factors. Using a similar methodology, Robertson et al (53) linked data from the Scottish Health Survey to mortality records and found similar results. Participants had a mean age of approximately 51 y at enrolment, and higher AL at enrolment was predictive of increased mortality ten years later. Using data from the 1958 British birth cohort, Castagne et al (60) found that higher AL at age 44 y was predictive of increased risk of mortality by age 55 y, even when accounting for early life experiences and both current and early life SES. By using death registry data to confirm death, Castagne et al (60) were able to include individuals who had been lost to follow up from the cohort in their mortality analysis.

1.4.5 Summary

Since its conceptualisation and operationalisation in the mid-1990s, research on allostatic load has illustrated its utility in predicting later adverse health outcomes and mortality, especially in older adults. Additional research has consistently linked higher AL scores with lower SES, and additional studies have found associations between adverse life events and early menarche and higher AL scores. Though there remains a lack of consensus about which biomarkers to include in an AL score and the mechanism of calculation from those biomarkers, AL has been shown to be a useful measure of overall physiological stress.

1.5 Literature Gaps

While considerable research has illustrated links between external factors, including early life growth, and individual adult health outcomes, limited research has been conducted on associations between external factors and AL in adolescents and young adults. Existing research has generally focused on associations between lower SES and higher AL, and these relationships have been found in both adolescents (52, 58) and adults (43, 51, 56). Very limited research has been conducted on associations between early life biological factors and adult AL, though the one study to look at this found associations between maternal BMI and age at birth with midlife allostatic load (59).

Furthermore, existing studies of allostatic load have generally been conducted in developed, upper-income countries, with little research conducted in low- or middle-income countries. External factors, especially in early life, may be very different in LMICs than in upper-income countries. South Africa in particular, where the data for this analysis was collected, was characterized by violence and rapid, social and political change coinciding with the dissolution of apartheid in the early 1990s during the early years of the participants' lives. As associations between early life growth and individual later life health outcomes have been illustrated in LMICs, examining if a link exists with a more comprehensive measure of health such as allostatic load can further clarify the long-term effects of early life growth and begin to integrate overall physiological health into the DOHaD framework.

1.6 Objectives

My study builds on and links the DOHaD and AL frameworks through using AL as a measure of adult health in the conceptual model outlined in Figure 1.1 and, to the best of my knowledge, is the first study to examine the association of growth in early life and general physiological health in early adulthood in the context of an upper middle-income country.

Using data from the Birth to Twenty Plus cohort in Soweto-Johannesburg I will:

1. Calculate a measure of allostatic load at age 22 y with and without the inclusion of a clinical measure of mental health status.
2. Determine the association of growth in early childhood, as measured by birthweight, conditional linear growth from 0-2 y and 2-5 y, and conditional weight gain from 0-2 y and 2-5 y with physiological health, as measured by allostatic load, at age 22 y.
3. Examine the mediating effect of adolescent body mass index and pubertal development trajectories on the association of early life growth and allostatic load at age 22 y.

Hypothesis:

Allostatic load score will be similar between the two sexes and, based on prior research linking early life growth with higher values of multiple AL components, higher AL will also be associated with increased growth in early life. BMI trajectories will likely play some mediating role in this relationship, while the potential role of pubertal trajectories is not as immediately clear.

Chapter Two – Submitted Manuscript

The manuscript that follows has been submitted to the Journal of Developmental Origins of Health and Disease on 7 March 2019.

2.1 Introduction

It is increasingly recognised that experiences and health in one stage of the life course can influence health outcomes in another stage. The 'developmental origins of health and disease' (DOHaD) framework posits that experiences in early life can result in permanent physiological changes that impact health factors later in life (1). Low birthweight has been associated with increased adult blood pressure, increased glucose intolerance, and increased risk of coronary heart disease (26, 29, 35). Similarly, rapid growth in childhood has been associated with increased risk of coronary heart disease and higher fat-free soft tissue mass in adulthood, and growth patterns in childhood have been associated with timing and tempo of puberty (16, 24, 26).

Beyond early childhood, considerable evidence suggests that increased adiposity throughout childhood and adolescence has adverse impacts on a range of adult health outcomes, including obesity, blood pressure, and risk of coronary heart disease (21, 27, 29, 30, 34). There is also evidence that pubertal factors influence adult health, as early adrenarche was found to be associated with higher risks of components of metabolic syndrome, such as type 2 diabetes, obesity, and cardiovascular disease, especially among females (28).

While links have been illustrated between early life and childhood growth and individual health outcomes, less is known about potential links to a more comprehensive measure of health. Contemporaneous with the development of the DOHaD framework, a composite measure of physiological stress known as allostatic load (AL) was being developed (11). As originally formulated, elevated AL results from chronic heightened responses of neural and neuroendocrine systems to perceived stresses, which then results in downstream effects on other biological systems, such as cardiometabolic and immune systems, and eventually adverse

disease outcomes (11). By combining measures of cardiovascular, metabolic, neuroendocrine, and immune health, a composite measure of physiological health can be calculated. Higher AL scores have been associated with adverse health outcomes in older adults (70+), as well as increased mortality among middle-aged adults (42, 53, 54, 60-62).

A recent review summarised evidence for a consistent association between lower socio-economic status (SES) and higher AL in adults, findings that have since been replicated in adolescents (52, 56). In women, early menarche has been shown to be associated with higher AL in early adulthood (55). In a Danish cohort, birth weight was found to be inversely associated with mid-life AL in females, though that study did not examine growth patterns in early life (59). However, these findings all come from developed, upper-income countries, and little to no research in this area has been conducted in low- or middle-income countries, where the context, particularly in early life, may be very different from upper-income countries. In the current analysis, we examine the association between growth in early life and allostatic load in age 22 y, as well as potential mediation of that relationship by adolescent body mass index or pubertal development trajectory, using data from the Birth to Twenty Plus (Bt20+) cohort in South Africa.

2.2 Methods

Study population

Data for this analysis come from the Birth to Twenty Plus study, a birth cohort consisting of 3273 singleton infants born in the Soweto-Johannesburg area of South Africa between April and June, 1990. A detailed description of the cohort, including recruitment and selection criteria, has been published previously (63). Most of the cohort resides in an urban, relatively poor community, and absolute attrition is relatively low at approximately 35% by age 28 y, though not all participants attended each study wave. Ethics approval for this analysis came from the University of the Witwatersrand Human Research Ethics Committee (Certificate #M180933) and participants or their caregivers, as appropriate, provided written informed consent at each study visit.

Only participants who returned for the age 22 y study visit were eligible for our analysis (n = 1552) (**Figure 2.1**). We excluded pregnant females (n = 22) and those females whose pregnancy status was not recorded at the time of the age 22 y study visit (n = 60). For calculations of AL, we excluded participants with C-reactive protein values ≥ 10 mg/L (n = 154), as such concentrations are indicative of an acute infection. Additionally, we excluded participants missing data on more than three allostatic load components (n = 276), resulting in a final sample for AL of 1036 participants. We excluded participants missing any early life growth measures (n = 437) or BMI/pubertal trajectory values (n = 51) for analyses involving those exposures, resulting in a final sample of 596 participants for those analyses.

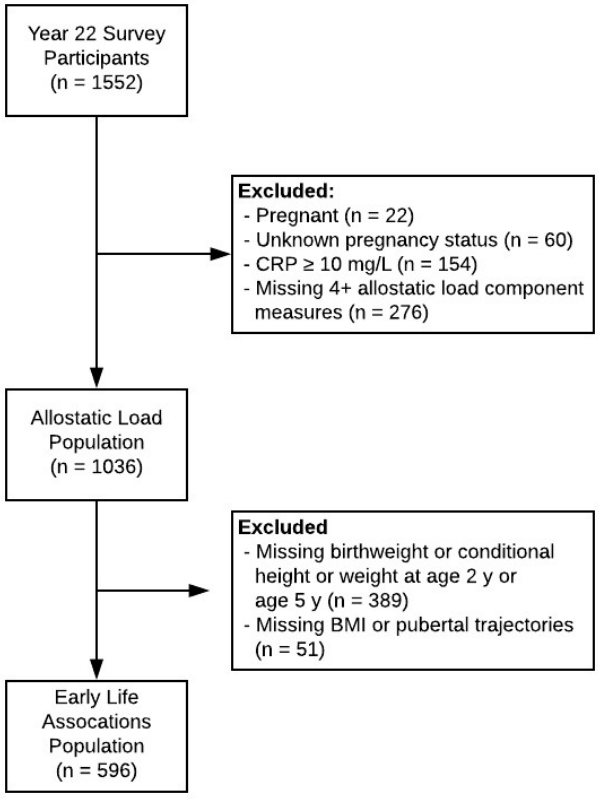


Figure 2.1: Consort flow diagram of sample size

Exposures

Birthweight was abstracted from birth notification records. Child height to the nearest 0.1 cm and weight to the nearest 0.1 kg were recorded by trained research staff using a wall-mounted stadiometer (Holtain, UK) and digital scale, respectively, at the age 2 y and age 5 y study visits. Conditional height given previous height and weight and conditional weight given current height and previous height and weight were computed as sex-specific residuals at age 2 y and 5 y (19). In essence, these variables are indicative of greater linear growth independent of weight and greater weight gain independent of height.

Mediators

Sex-specific trajectories of body-mass index (BMI) from age 5 y to 18 y were previously computed using latent class growth mixture modelling for participants with

at least two BMI measurements between age 5 y and 18 y (Supplemental Figure S1). Three trajectories were identified for males (1 – normal weight; 2 – early onset overweight to normal; 3 – early onset overweight to obese) and four trajectories were identified for females (1 – normal weight; 2 – early onset obese to overweight; 3 – early onset obese to morbidly obese; 4 – late onset overweight) (30). Latent class growth analysis was previously used with serial Tanner sexual maturation scale measurements from age 9 y to 16 y to calculate sex-specific trajectories of pubertal development. These trajectories capture information regarding both the timing of pubertal onset and rate of progression through puberty. Three trajectories of pubic hair development were identified for both males and females, with four trajectories of breast development identified for females and four trajectories of genital development identified for males (Supplemental Figure S2). For all trajectories, trajectory one represents participants with latest onset of puberty and slowest development, with increasing trajectory numbers representing successively earlier onset and faster development (16).

Outcome

We created an overall measure of allostatic load from eleven health measures collected at the age 22 y study visit. Measures were selected to encompass multiple physiological systems and the choice of which measures to include was based on literature examples and data availability (45, 47, 52, 53, 55, 58, 64, 65). We used systolic blood pressure (SBP), diastolic blood pressure (DBP), and resting heart rate as measures of cardiovascular health. Markers of metabolic health included BMI, waist-to-hip ratio (WHR), total cholesterol, high density lipoprotein (HDL), triglycerides, and fasting blood glucose. C-reactive protein (CRP) was included as a marker of inflammation. Total score from the 28-item General Health Questionnaire (GHQ-28), in which patients assess changes in their mood, feelings, and behaviours over the last four weeks, was included as a marker of psychological distress (66).

Height, weight, waist size, hip size, and resting heart rate were recorded by trained research staff during the age 22 y study visit, with a coefficient of variation of < 1% for repeated measures. Blood pressure was measured in triplicate with participants in a seated position using an Omron M6 (Kyoto, Japan), with the mean of the second and third measurements used for analysis. Total cholesterol, HDL,

triglycerides, fasting blood glucose, and CRP were measured from venous blood draws collected following an overnight fast. Plasma glucose was measured by an autoanalyzer using standard enzymatic methods, blood lipids were measured by standard enzymatic methods, and CRP concentrations were measured using a full range CRP immunoturbidimetric assay (Randox Laboratories; South Africa). Quality was checked by control samples and the coefficient of variation for lab measures was < 2%.

To create the AL index, we created empirical cut points for each health measure based on the sample distribution. For all measures except HDL, an observed level above the 75th percentile was regarded as high-risk, and for HDL an observed level below the 25th percentile was regarded as high-risk. A dichotomous indicator was created for each health measure, with a value of 1 assigned for high-risk values and a value of 0 assigned to low-risk values. The indicator variables were summed to create an overall measure of AL for each participant, ranging from 0 to 11. Based on the distribution of the resulting allostatic load measure, we created an indicator for 'high AL' defined as AL > 4, which included ~ 15% of the study sample.

Confounders

Confounders were identified *a priori* based on literature driven hypothesized relationships with both the exposure variables and the AL outcome. Gestational age, maternal age and education, parity, and household asset ownership from age 0-2 y (as a proxy for socioeconomic status), all of which come from the original Bt20+ enrolment data, were included as confounders.

Missing Data

265 participants (26%) were missing data for one to three AL component measures, with the majority of those (239; 90%) missing a single measure. In order to include these participants in the analyses, missing values for these measures, along with missing confounder values, were imputed using multiple imputation by chained equations. Missing individual component measures, rather than the overall AL score, were imputed to utilize the observed values of the other AL component measures. Empirical cut points for each AL component, high-risk indicators, and a summary measure of AL were calculated within each imputed dataset as described previously. Participants missing greater than three AL components were generally

missing an entire suite of measures (e.g. all bloodwork related measures) and were therefore excluded. Including missing confounders, 41% of participants had a least one value imputed, and we therefore imputed 50 datasets (67).

Statistical Analysis

We examined differences in the percentage of individuals with high-risk values for each AL component measure and the percentage of individual with high AL by sex using unadjusted pooled logistic regression across the imputed datasets. Differences in the distribution of AL score by sex were examined using unadjusted pooled Poisson regression. We examined the association between early life growth measures and age 22 y AL score using sex-specific unadjusted and adjusted pooled Poisson regression. Potential mediation of the association between early life growth and age 22 y AL by BMI trajectories and/or pubertal trajectories was assessed by adding each type of trajectory to the model both individually and in conjunction with the other trajectories and assessing the resulting change in association between early life growth and age 22 y AL. In addition to examining the associations with the count AL measure, we examined the same associations with the indicator for high AL using sex-specific unadjusted and adjusted pooled logistic regression.

We utilised Poisson regression for the analyses with AL score because AL is a discrete, non-negative outcome. We explored the use of negative-binomial models to allow for additional dispersion in the outcome variable but found no evidence of overdispersion and consequently used Poisson models.

Sensitivity Analyses

To investigate potential selection effects, we compared demographics of participants included in our analyses to those who were originally enrolled in Bt20+ but were excluded from the current study. As GHQ-28 is not a measure of physiological health and has not been included in previous characterizations of AL, we compared our results to a calculation of AL that did not include GHQ-28 as a component measure. In addition to the sex-specific analyses, we ran pooled analyses controlling for sex to investigate the effect of the larger sample size on variance in the model estimates. All analyses were conducted using R version 3.5.2, with multiple imputation by chained equations done using the ‘mice’ package (68, 69).

2.3 Results

Early life anthropometric data, maternal and household characteristics, and pubertal trajectory memberships for the included sample are displayed in **Table 2.1**. Most participants are in BMI trajectory 1, the 'normal weight' trajectory for both sexes, while there was more variation in membership in the pubertal trajectories.

Participants included in the AL calculation were more likely to be Black than excluded participants, which is a result of the increased emphasis on recruitment of Black participants at the age 22 visit (Supplemental Table S1). Participants included in the analyses of associations between AL and early life growth were more likely to have shorter gestational age, lower maternal parity, lower maternal age, and higher asset score at 0-2 y than participants who only had an AL score calculated; all factors were controlled for in the regression models (Supplemental Table S2). As BMI trajectories were only developed for Black participants, our analysis of associations between growth measures and age 22 y AL was restricted to Black participants.

Table 2.2 shows the high-risk cut point values, the number of missing values imputed, and the mean number of participants with elevated values of each AL component measure by sex. We found significant differences in the percentage of males and females with high-risk values for many component measures, with males being more likely to have high-risk values of SBP, DBP, WHR, triglycerides, fasting blood glucose, and CRP and females more likely to have high-risk values of resting heart rate, BMI, total cholesterol, HDL, and GHQ-28 score. When including GHQ-28 in the calculation of the summary AL score, females had a higher average AL score (2.91) than males (2.66), though this difference did not remain when excluding GHQ-28 from the AL score calculation. There were no differences in the percentages of males and females with high AL in either calculation of AL. The distribution of AL scores by sex in a single imputed dataset is shown in **Figure 2.2**, and distributions across the remaining imputed datasets were qualitatively similar.

There was no consistent association between any early life growth measure and allostatic load at age 22 y in males or females in the adjusted analyses (**Table 2.3**). Unadjusted results are presented in Supplemental Table S3. Conditional weight gain from 2-5 y appeared marginally associated with age 22 y AL in males but did

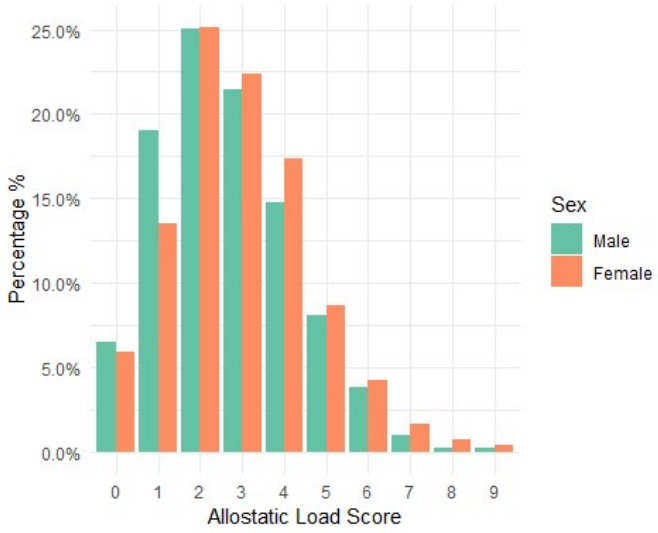


Figure 2.2: Percentage distribution of calculated age 22 y allostatic load score in the first imputation by sex, Birth to Twenty Plus cohort (n = 1036)

not reach statistical significance. We found no significant associations between either BMI trajectory or pubertal development trajectory and age 22 y AL among males when controlling for early life growth, while among females, BMI trajectories 3 and 4 were significantly associated with increased age 22 y AL compared to the normal BMI trajectory (Table 2.3, Models 2-4). These trajectories correspond to 'early onset obese to morbidly obese' (trajectory 3) and 'late onset overweight' (trajectory 4). When pooling males and females together and adjusting for sex, there remained no association between any early life growth measure and age 22 y AL (Supplemental Table S4). Associations with the version of AL that excluded GHQ-28 as a component measure were qualitatively similar to the main results (Supplemental Table S5).

Among males, higher conditional change in weight from 2-5 y was significantly associated with greater odds of high AL at age 22 y, and this relationship was not mediated by inclusion of either the BMI or pubertal trajectories (**Table 2.4**). Among females, no early life growth measures were associated with high AL at age 22 y, though membership in BMI trajectory 3 ('early onset obese to morbidly obese') was consistently associated with higher odds of high AL at age 22 y. These results were similar in unadjusted models (Supplemental Table S6). When considering the version of AL excluding GHQ-28, conditional weight gain from 2-5 y remained associated with high AL in males and conditional height gain from 2-5 y was associated with high AL in females, with evidence that this association is mediated through BMI trajectories (Supplemental Table S7). When pooling males and females together and adjusting for sex, only the association of BMI trajectory 3 with high AL remained significant (Supplemental Table S8).

Table 2.1: Early life anthropometric measures and maternal/household characteristics by sex, Birth to Twenty Plus cohort (n = 1036)

	Males (n = 495)		Females (n = 541)	
	N (%)	**Mean (SD)**	**N (%)**	**Mean (SD)**
Birthweight (kg)	494	3.12 (0.51)	541	3.02 (0.49)
Height at age 2 (cm)	330	83.37 (3.37)	388	82.74 (3.21)
Height at age 5 (cm)	308	107.83 (4.56)	367	107.18 (4.52)
Weight at age 2 (kg)	396	11.46 (1.41)	451	11.24 (1.25)
Weight at age 5 (kg)	375	18.21 (2.13)	432	17.88 (2.33)
Gestational age (weeks)	485	38.03 (1.8)	533	37.95 (1.96)
Maternal education	456	9.41 (2.66)	508	9.66 (2.73)
Maternal age (years)	495	26.09 (6.43)	540	25.89 (6.06)
Maternal parity	495	2.4 (1.56)	541	2.19 (1.32)
Asset score 0-2 years	395	3.51 (1.69)	452	3.42 (1.63)
Ethnicity				
- Black	468 (95%)		505 (93%)	
- Other[1]	27 (5%)		36 (7%)	
Childhood/adolescent BMI trajectory[2]				
- 1	441 (89%)		411 (76%)	
- 2	21 (4%)		21 (4%)	
- 3	5 (1%)		19 (4%)	
- 4	-		51 (9%)	
- Missing	28 (6%)		39 (7%)	
Pubic hair trajectory[3]				
- 1	132 (27%)		130 (24%)	
- 2	293 (59%)		326 (60%)	
- 3	68 (14%)		80 (15%)	
- Missing	2 (0%)		5 (1%)	
Breast/genital development trajectory[3]				
- 1	122 (25%)		87 (16%)	
- 2	189 (38%)		161 (30%)	
- 3	131 (26%)		164 (30%)	
- 4	51 (10%)		125 (23%)	
- Missing	2 (0%)		4 (1%)	

[1] 'Other' includes White, Coloured, and Indian

[2] BMI trajectory definitions: 1 – normal weight; 2 – early onset overweight to normal weight (males) or early onset obese to overweight (females); 3 – early onset overweight to obese (males) or early onset obese to morbidly obese (females); 4 – late onset overweight (females only)

[3] Trajectory 1 represents children who started puberty late and progressed slowly, with higher trajectories having progressively earlier pubertal start and faster tempo.

Table 2.2: Descriptive statistics of allostatic load component measures and summary values by sex, including number of observations imputed for each measure, the mean and range of high-risk cutoff value, the mean percentage of participants with component measure values in the high-risk quartile, and the mean allostatic load score, all calculated across imputations.

	Obs. Imputed	Mean high-risk cutoff value (range)	Males (n = 495)	Females (n = 541)	p[1]
Cardiovascular Markers					
Systolic blood pressure (mm Hg)	1	121 (121, 121)	39%	12%	< 0.001
Diastolic blood pressure (mm Hg)	1	78 (78, 78)	30%	23%	0.015
Resting heart rate (bpm)	1	79.5 (79.5, 79.5)	8%	42%	< 0.001
Metabolic Markers					
Body mass index (kg/m^2)	8	25.84 (25.82, 25.87)	10%	39%	< 0.001
Waist-to-hip ratio	49	0.83 (0.83, 0.83)	31%	20%	< 0.001
Total cholesterol (mmol/L)	10	3.87 (3.87, 3.88)	16%	34%	< 0.001
High density lipoprotein (mmol/L)	3	0.95 (0.95, 0.95)	22%	28%	0.045
Triglycerides (mmol/L)	3	0.66 (0.66, 0.66)	33%	18%	< 0.001
Fasting glucose (mmol/L)	20	5.20 (5.19, 5.21)	30%	21%	0.001
Inflammation Markers					
C-reactive protein (mg/L)	170	3.64 (3.54, 3.78)	29%	21%	0.013
Emotional Distress					
GHQ-28 score	30	25 (25, 25)	18%	33%	< 0.001
Allostatic Load					
Including GHQ-28	-	-	2.66	2.91	0.019
Excluding GHQ-28	-	-	2.48	2.58	0.307
High Allostatic Load (> 4)					
Including GHQ-28	-	-	13%	16%	0.253
Excluding GHQ-28	-	-	11%	12%	0.766

[1] P-values calculated using pooled logistic regression (component measures and high allostatic load) and pooled Poisson regression (allostatic load score).

Table 2.3: Associations of age 22 y allostatic load with early life growth as potentially mediated by childhood/adolescent body mass index and adolescent pubertal trajectories in the Birth to Twenty Plus cohort (n = 596). Values displayed are adjusted risk ratios (95% CI) for a 1 unit increase in allostatic load measures estimated by Poisson regression.[1]

	Males (n = 282)				Females (n = 314)			
	Model 1	Model 2	Model 3	Model 4	Model 1	Model 2	Model 3	Model 4
Birthweight	1.05 (0.89, 1.22)	1.06 (0.91, 1.24)	1.05 (0.90, 1.23)	1.07 (0.91, 1.25)	1.02 (0.87, 1.20)	1.02 (0.86, 1.20)	1.03 (0.87, 1.22)	1.03 (0.87, 1.21)
Conditional height 0-2 y	0.98 (0.91, 1.06)	0.99 (0.92, 1.07)	0.98 (0.91, 1.06)	0.99 (0.92, 1.07)	1.03 (0.96, 1.11)	1.03 (0.96, 1.11)	1.02 (0.94, 1.09)	1.02 (0.95, 1.10)
Conditional height 2-5 y	1.04 (0.97, 1.12)	1.03 (0.95, 1.11)	1.04 (0.96, 1.12)	1.03 (0.95, 1.11)	1.06 (0.98, 1.14)	1.03 (0.96, 1.12)	1.05 (0.97, 1.14)	1.03 (0.95, 1.11)
Conditional weight 0-2 y	1.06 (0.99, 1.14)	1.06 (0.99, 1.13)	1.06 (0.99, 1.13)	1.06 (0.99, 1.13)	1.00 (0.93, 1.07)	1.01 (0.94, 1.08)	0.99 (0.93, 1.07)	1.00 (0.93, 1.08)
Conditional weight 2-5 y	1.08 (1.00, 1.16)	1.08 (1.00, 1.16)	1.08 (1.00, 1.16)	1.08 (1.00, 1.16)	1.03 (0.96, 1.10)	1.01 (0.95, 1.08)	1.02 (0.95, 1.09)	1.00 (0.94, 1.07)
BMI trajectory[2]								
- 1	X	Ref	X	Ref	X	Ref	X	Ref
- 2	X	1.17 (0.83, 1.65)	X	1.16 (0.82, 1.64)	X	1.23 (0.88, 1.72)	X	1.20 (0.86, 1.68)
- 3	X	1.66 (0.92, 2.98)	X	1.63 (0.90, 2.94)	X	1.51 (1.11, 2.04)	X	1.47 (1.08, 2.00)
- 4	-	-	-	-	X	1.23 (1.00, 1.52)	X	1.26 (1.02, 1.55)
Pubic hair trajectory[3]								
- 1	X	X	Ref	Ref	X	X	Ref	Ref
- 2	X	X	1.05 (0.81, 1.36)	1.04 (0.80, 1.35)	X	X	1.12 (0.94, 1.33)	1.14 (0.96, 1.36)
- 3	X	X	1.01 (0.71, 1.43)	1.00 (0.71, 1.42)	X	X	1.09 (0.84, 1.41)	1.11 (0.85, 1.44)
Breast/genital development trajectory[3]								
- 1	X	X	Ref	Ref	X	X	Ref	Ref
- 2	X	X	0.99 (0.76, 1.29)	0.98 (0.75, 1.28)	X	X	0.94 (0.76, 1.16)	0.90 (0.73, 1.12)
- 3	X	X	1.02 (0.75, 1.38)	1.00 (0.74, 1.35)	X	X	0.94 (0.75, 1.18)	0.92 (0.73, 1.15)
- 4	X	X	0.94 (0.65, 1.37)	0.94 (0.65, 1.36)	X	X	1.09 (0.85, 1.39)	1.03 (0.80, 1.32)

[1] All models adjusted for gestational age, maternal age, maternal years of education, parity, and age 0-2y physical asset score

[2] BMI trajectory definitions: 1 – normal weight; 2 – early onset overweight to normal weight (males) or early onset obese to overweight (females); 3 – early onset overweight to obese (males) or early onset obese to morbidly obese (females); 4 – late onset overweight (females only)

[3] Trajectory 1 represents children who started puberty late and progressed slowly, with higher numbered trajectories having progressively earlier pubertal start and faster tempo.

Table 2.4: Sex-specific associations of high (> 4) age 22 y allostatic load with early life growth as potentially mediated by childhood/adolescent body mass index and adolescent pubertal trajectories in the Birth to Twenty Plus cohort (n = 596). Values displayed are adjusted odds ratios (95% CI) estimated by logistic regression.[1]

	Males (n = 282)				Females (n = 314)			
	Model 1	Model 2	Model 3	Model 4	Model 1	Model 2	Model 3	Model 4
Birthweight	1.35 (0.57, 3.16)	1.43 (0.60, 3.40)	1.32 (0.55, 3.16)	1.39 (0.58, 3.34)	0.95 (0.43, 2.08)	0.92 (0.41, 2.05)	0.96 (0.43, 2.15)	0.93 (0.41, 2.10)
Conditional height 0-2 y	0.90 (0.60, 1.34)	0.94 (0.62, 1.41)	0.90 (0.60, 1.35)	0.94 (0.62, 1.43)	1.16 (0.82, 1.64)	1.20 (0.83, 1.73)	1.11 (0.77, 1.58)	1.14 (0.78, 1.67)
Conditional height 2-5 y	1.25 (0.82, 1.89)	1.13 (0.73, 1.75)	1.24 (0.81, 1.90)	1.13 (0.73, 1.77)	1.40 (0.97, 2.02)	1.28 (0.86, 1.89)	1.37 (0.94, 2.00)	1.25 (0.84, 1.87)
Conditional weight 0-2 y	1.11 (0.78, 1.59)	1.10 (0.76, 1.58)	1.10 (0.76, 1.58)	1.09 (0.75, 1.58)	1.27 (0.91, 1.78)	1.33 (0.94, 1.90)	1.24 (0.89, 1.75)	1.32 (0.92, 1.88)
Conditional weight 2-5 y	1.88 (1.19, 2.98)	1.83 (1.13, 2.98)	1.90 (1.18, 3.05)	1.84 (1.12, 3.03)	0.99 (0.72, 1.37)	0.90 (0.65, 1.26)	0.95 (0.69, 1.32)	0.87 (0.62, 1.23)
BMI trajectory[2]								
- 1	X	Ref	X	Ref	X	Ref	X	Ref
- 2	X	2.47 (0.55, 11.02)	X	2.54 (0.55, 11.83)	X	2.69 (0.62, 11.69)	X	2.41 (0.55, 10.64)
- 3	X	7.02 (0.45, 108.87)	X	7.09 (0.44, 115.06)	X	5.31 (1.39, 20.32)	X	4.90 (1.23, 19.45)
- 4	X	X	X	-	X	2.41 (0.93, 6.29)	X	2.59 (0.97, 6.90)
Pubic hair trajectory[3]								
- 1	X	X	Ref	Ref	X	X	Ref	Ref
- 2	X	X	0.80 (0.19, 3.40)	0.73 (0.17, 3.14)	X	X	1.20 (0.50, 2.87)	1.31 (0.53, 3.21)
- 3	X	X	1.09 (0.18, 6.64)	1.02 (0.17, 6.14)	X	X	1.22 (0.35, 4.18)	1.35 (0.37, 4.92)
Breast/genital development trajectory[3]								
- 1	X	X	Ref	Ref	X	X	Ref	Ref
- 2	X	X	0.95 (0.22, 4.04)	0.95 (0.22, 4.07)	X	X	0.90 (0.31, 2.64)	0.76 (0.25, 2.33)
- 3	X	X	1.42 (0.28, 7.18)	1.36 (0.27, 6.92)	X	X	0.92 (0.29, 2.86)	0.83 (0.26, 2.68)
- 4	X	X	0.93 (0.13, 6.66)	0.97 (0.14, 6.73)	X	X	1.58 (0.49, 5.15)	1.23 (0.36, 4.25)

[1] All models adjusted for gestational age, maternal age, maternal years of education, parity, and age 0-2y physical asset score

[2] BMI trajectory definitions: 1 – normal weight; 2 – early onset overweight to normal weight (males) or early onset obese to overweight (females); 3 – early onset overweight to obese (males) or early onset obese to morbidly obese (females); 4 – late onset overweight (females only)

[3] Trajectory 1 represents children who started puberty late and progressed slowly, with higher numbered trajectories having progressively earlier pubertal start and faster tempo.

2.4 Discussion

Our study profiled the varying components of AL among a cohort of South African young adults and explored potential relationships between AL and growth earlier in life. We found significant differences between males and females with regards to the components of the AL score, though sex differences in the distributions of the final AL score were small. Increased weight gain from ages 2-5 y were consistently associated with greater odds of high (> 4) AL among males, with evidence for an association between increased height gain from 2-5 y and high AL among females mediated by BMI trajectory when considering only the physical components of AL score. Unhealthy adolescent BMI trajectories were consistently associated with higher AL scores.

The idea of a comprehensive measure of physiological health is an appealing one. AL aims to incorporate measures related to multiple body systems and combine them into a single quantity that reflects cumulative 'wear and tear' on the body (11, 42). Such a composite risk factor sacrifices information on particular indicators, though previous research has illustrated the predictive value of AL for other morbidities and mortality in middle-aged and older adults (42, 53, 54, 60-62). While we found significant differences between the proportion of males and females with 'high-risk' values for most AL components, we found only minimal differences in the distribution of AL and no sex differences in the percentage of participants with high AL. Consistent with the AL framework, this suggests that while males and females in our study population had similar 'wear and tear,' this was expressed via different indicators for each sex, and a focus on any particular indicator might give an incomplete picture of overall health. Further research will help elucidate whether AL in early adulthood will have similar predictive value for future adult health and morbidity.

Among early life measures, only increased conditional weight gain from 2-5 y among males was associated with greater odds of high age 22 y AL. When GHQ-28 was excluded from the calculation of AL, we found an association between increased conditional height gain from 2-5y among females and greater odds of high AL, with evidence that this association was mediated through unhealthy childhood/adolescent BMI trajectories. These findings were supported in the analyses of continuous AL

score, though the results did not reach the level of statistical significance. Previous research illustrated links between early life growth and adult blood pressure, body composition, and diabetes risk, and components related to each of those outcomes are included in our calculation of AL (29, 31, 35, 70). Our study suggests that while increased growth from 2-5y may be associated with multiple morbidities as expressed through high AL score, there is not a consistent association between early life growth and increased morbidity in early adulthood. We found consistent associations between BMI trajectories corresponding to 'early onset overweight/obese to obese/morbidly obese' and 'late onset obese' and poor AL outcomes at age 22 y. While these associations were clearly present among young women and in the pooled analysis, the small number of men in the high BMI trajectories makes it more challenging to draw specific conclusions for males. These results are consistent with research that has demonstrated associations between higher BMI in childhood and adolescence and increased risk of coronary heart disease, obesity, high blood pressure, diabetes, and premature mortality (21, 27, 29, 34).

Strengths of our current research include the longitudinal nature of the data, allowing us to use measures collected in childhood and adolescence and directly link those with young adult measures in the same individual. Retention in the Bt20+ cohort is high from year to year, providing us with a relatively large sample. By using multiple imputation, we were able to utilize partial data from hundreds of participants that would have been discarded in a complete case analysis. As the cohort is still active, later rounds of data collection will allow for examinations of associations of early life growth with AL later in life or examinations of how AL in early adulthood may predict future health outcomes.

Our study is not without limitations. We calculated AL using data from the age 22 y study wave, which is early in adulthood and potentially prior to the occurrence of the adverse health outcomes that have been documented in other DOHaD studies. However, the empirical high-risk quartile cut points for multiple components were close to their relevant clinical values, indicating that morbidities for at least some AL components are already present in our study population. The summary AL index used equally weights all components, which is unlikely to accurately reflect contributions of the different AL components to health. The summary index is a

commonly used approach to calculating AL in the literature, and earlier research examining alternative AL calculation methods such as factor analysis found that simple summations performed comparably to more complex methods (42).

Using data from a longitudinal birth cohort in urban South Africa, we found similar levels of AL in males and females at age 22 y, though the components contributing to the AL score varied by sex. While AL may be a useful tool to identify young adults at risk of future health issues, further longitudinal research is needed to determine any links between young adult AL and future health. Unhealthy BMI trajectories were associated with both increased continuous AL scores and high AL. This highlights the potential early signs of continuing physiological risk due to higher adiposity in childhood and adolescence. While earlier DOHaD literature has illustrated relationships between early life growth and several components of our AL measure, we only found consistent associations between conditional weight gain from 2-5 y and high AL among males. Our study expands the DOHaD literature by going beyond individual health markers to consider a comprehensive measure of physiological health and suggests that early life growth is not highly associated with such an overall measure of health. Future work can expand on our research by considering measures of AL at later ages and investigating associations between young adult AL and later adverse health events.

2.5 Supplemental Figures and Tables

Figure S1: Body mass index trajectories in females (A) and males (B) from age 5 to 18y, Birth to Twenty Plus cohort. Trajectories for both sexes are plotted along with Extended International Obesity Task Force Cut-Offs for overweight, obesity, and morbid obesity. Reprinted from Munthali et al 2016 (30).

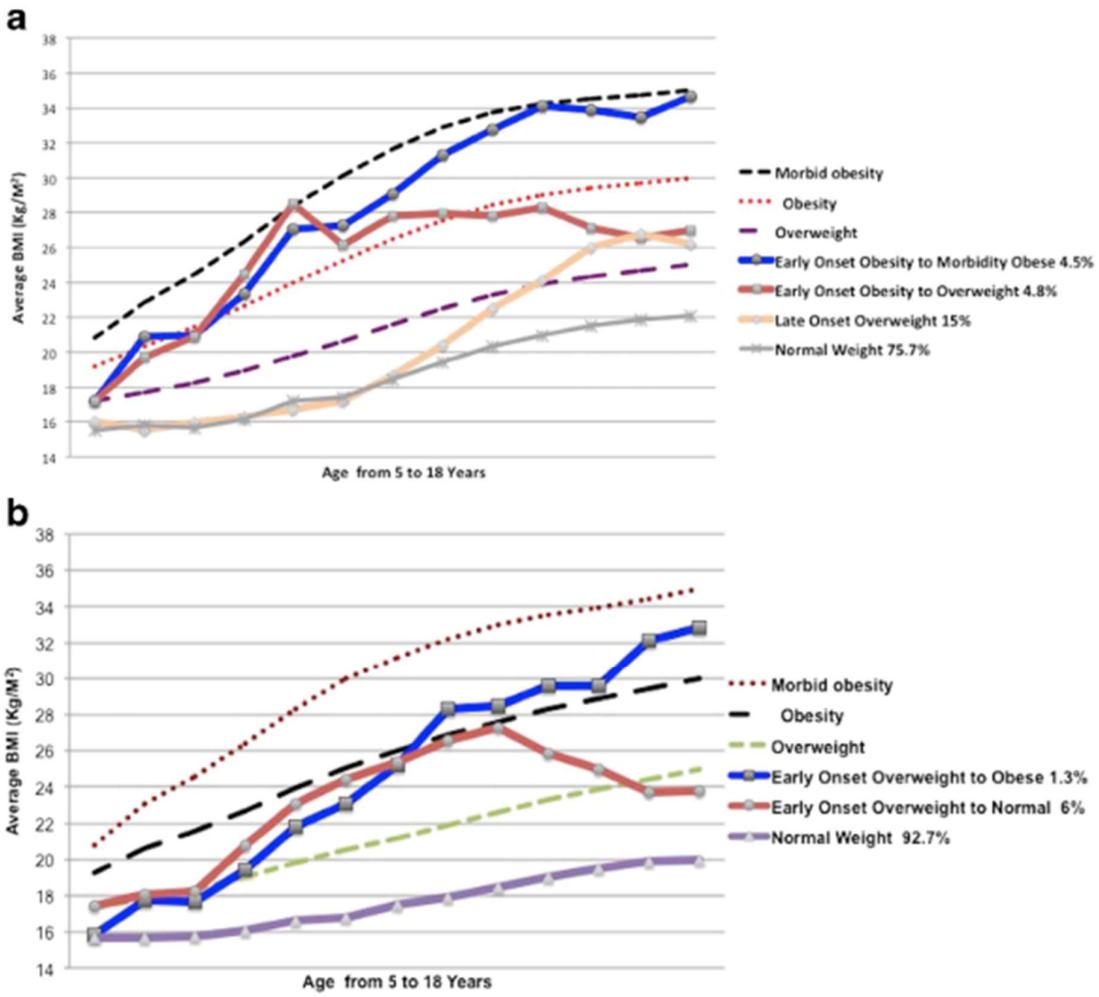


Figure S2: Mean Tanner sexual maturity scale score by trajectory of pubertal development from ages 9 to 16 y, Birth to Twenty Plus cohort. A) female pubic hair development; B) female breast development; C) male pubic hair development; D) male genital development. Reprinted from Lundeen et al 2016 (16).

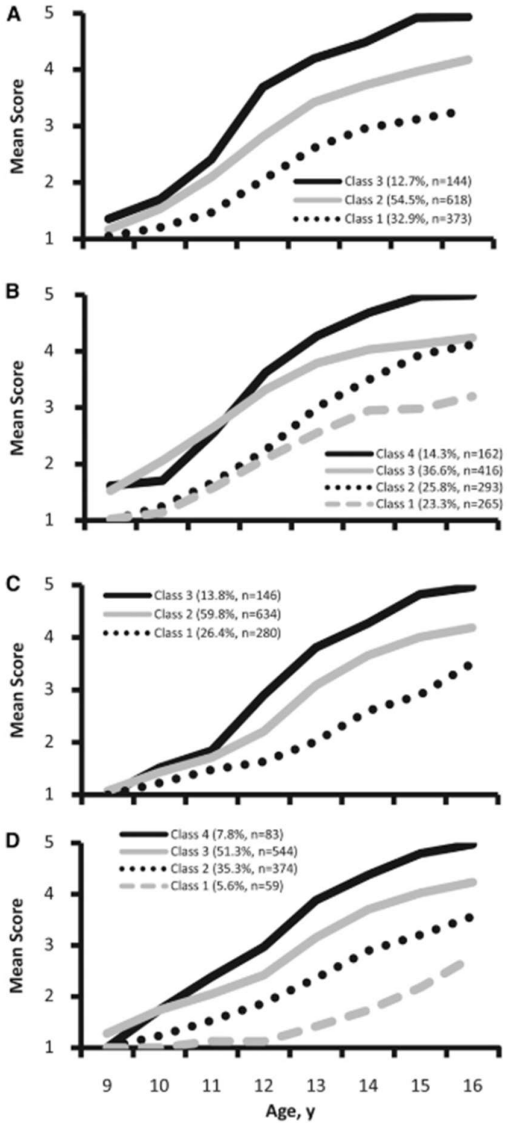


Table S1: Early life, maternal, and household characteristics by study participation status, Birth to Twenty Plus cohort (n = 3273). Values shown are mean (standard deviation) or n (%), with p-values calculated using Kruskal-Wallis non-parametric ANOVA for continuous variables and a χ^2 test for categorical variables

	Excluded from AL sample (n = 2237)	Included in AL sample (n = 1036)	p
Birthweight (kg)	3.07 (0.52)	3.07 (0.5)	0.863[1]
Gestational age (weeks)	38.22 (1.93)	37.99 (1.88)	< 0.001
Maternal parity	2.23 (1.35)	2.29 (1.44)	0.539
Maternal age (years)	25.95 (6.01)	25.99 (6.24)	0.839
Maternal education (years)	9.56 (3.14)	9.54 (2.7)	0.475
Asset score 0-2 years	3.68 (1.98)	3.46 (1.66)	0.012
Sex			
- Male	1099 (49%)	495 (48%)	0.496
- Female	1138 (51%)	541 (52%)	
Ethnicity			
- Black	1595 (71%)	973 (94%)	< 0.001
- Other[2]	642 (29%)	63 (6%)	
Small for gestational age			
- No	1832 (85%)	883 (87%)	0.235
- Yes	319 (15%)	134 (13%)	

[1] Significance assessed by ANOVA due to normal distribution of outcome

[2] 'Other' includes White, Coloured, and Indian

Table S2: Early life, maternal, and household characteristics among those with allostatic load measures by inclusion/exclusion from early life regression models, Birth to Twenty Plus cohort (n = 1036). Values shown are mean (standard deviation) or n (%), with p-values calculated using Kruskal-Wallis non-parametric ANOVA for continuous variables and a χ^2 test for categorical variables

	Excluded from regression models (n = 440)	Included in regression models (n = 596)	p
Birthweight (kg)	3.06 (0.49)	3.07 (0.51)	0.644[1]
Gestational age (weeks)	38.17 (1.94)	37.86 (1.83)	< 0.001
Maternal parity	2.45 (1.5)	2.17 (1.39)	< 0.001
Maternal age (years)	26.52 (5.95)	25.6 (6.42)	0.006
Maternal education (years)	9.44 (2.82)	9.61 (2.61)	0.369
Asset score 0-2 years	3.12 (1.76)	3.65 (1.57)	< 0.001
Sex			
- Male	213 (48%)	282 (47%)	0.775
- Female	227 (52%)	314 (53%)	
Ethnicity			
- Black	377 (86%)	596 (100%)	< 0.001
- Other[2]	63 (14%)	0 (0%)	
Small for gestational age			
- No	366 (86%)	517 (87%)	0.638
- Yes	59 (14%)	75 (13%)	

[1] Significance assessed by ANOVA due to normal distribution of outcome

[2] 'Other' includes White, Coloured, and Indian

Table S3: Unadjusted associations of age 22 y allostatic load score with early life growth as potentially mediated by childhood/adolescent body mass index and adolescent pubertal trajectories in the Birth to Twenty Plus cohort (n = 596). Values displayed are unadjusted risk ratios (95% CI) for a 1 unit increase in allostatic load measures estimated by Poisson regression.

	Males (n = 282)				Females (n = 314)			
	Model 1	Model 2	Model 3	Model 4	Model 1	Model 2	Model 3	Model 4
Birthweight	1.01 (0.88, 1.17)	1.03 (0.89, 1.19)	1.02 (0.88, 1.18)	1.03 (0.89, 1.20)	1.04 (0.91, 1.19)	1.03 (0.90, 1.18)	1.04 (0.91, 1.20)	1.04 (0.90, 1.19)
Conditional height 0-2 y	0.98 (0.91, 1.06)	0.99 (0.92, 1.07)	0.98 (0.91, 1.06)	0.99 (0.92, 1.07)	1.03 (0.96, 1.10)	1.03 (0.96, 1.11)	1.02 (0.94, 1.09)	1.02 (0.95, 1.10)
Conditional height 2-5 y	1.04 (0.96, 1.12)	1.02 (0.94, 1.10)	1.03 (0.96, 1.11)	1.02 (0.94, 1.10)	1.06 (0.98, 1.14)	1.04 (0.96, 1.12)	1.05 (0.97, 1.14)	1.03 (0.95, 1.11)
Conditional weight 0-2 y	1.07 (1.00, 1.15)	1.07 (0.99, 1.14)	1.07 (1.00, 1.14)	1.07 (0.99, 1.14)	1.00 (0.93, 1.07)	1.01 (0.94, 1.08)	0.99 (0.92, 1.06)	1.00 (0.93, 1.07)
Conditional weight 2-5 y	1.07 (0.99, 1.15)	1.07 (0.99, 1.15)	1.07 (0.99, 1.15)	1.07 (0.99, 1.15)	1.03 (0.96, 1.09)	1.01 (0.95, 1.08)	1.02 (0.95, 1.09)	1.00 (0.94, 1.07)
BMI trajectory[1]								
- 1	X	Ref	X	Ref	X	Ref	X	Ref
- 2	X	1.19 (0.85, 1.66)	X	1.18 (0.84, 1.66)	X	1.24 (0.89, 1.73)	X	1.21 (0.87, 1.69)
- 3	X	1.76 (0.99, 3.13)	X	1.73 (0.97, 3.09)	X	1.52 (1.12, 2.04)	X	1.48 (1.09, 2.01)
- 4	-	-	-	-	X	1.23 (1.00, 1.51)	X	1.26 (1.02, 1.55)
Pubic hair trajectory[2]								
- 1	X	X	Ref	Ref	X	X	Ref	Ref
- 2	X	X	1.07 (0.83, 1.39)	1.06 (0.82, 1.37)	X	X	1.12 (0.94, 1.33)	1.14 (0.96, 1.36)
- 3	X	X	1.05 (0.74, 1.48)	1.04 (0.74, 1.47)	X	X	1.09 (0.84, 1.41)	1.11 (0.86, 1.45)
Breast/genital development trajectory[2]								
- 1	X	X	Ref	Ref	X	X	Ref	Ref
- 2	X	X	0.97 (0.75, 1.26)	0.97 (0.74, 1.26)	X	X	0.94 (0.76, 1.16)	0.90 (0.73, 1.12)
- 3	X	X	0.99 (0.73, 1.34)	0.98 (0.72, 1.32)	X	X	0.94 (0.75, 1.18)	0.92 (0.74, 1.16)
- 4	X	X	0.91 (0.63, 1.32)	0.91 (0.63, 1.31)	X	X	1.09 (0.85, 1.39)	1.02 (0.80, 1.31)

[1] BMI trajectory definitions: 1 – normal weight; 2 – early onset overweight to normal weight (males) or early onset obese to overweight (females); 3 – early onset overweight to obese (males) or early onset obese to morbidly obese (females); 4 – late onset overweight (females only)

[2] Trajectory 1 represents children who started puberty late and progresses slowly, with higher trajectories having progressively earlier pubertal start and faster tempo.

Table S4: Adjusted associations of age 22 y allostatic load with early life growth as potentially mediated by childhood/adolescent body mass index and adolescent pubertal trajectories in the Birth to Twenty Plus cohort (n = 596). Values displayed are adjusted risk ratios (95% CI) for a 1 unit increase in allostatic load measures estimated by Poisson regression.[1]

	Model 1	Model 2	Model 3	Model 4
Birthweight	1.04 (0.93, 1.16)	1.04 (0.93, 1.16)	1.04 (0.93, 1.17)	1.04 (0.93, 1.17)
Conditional height 0-2 y	1.00 (0.95, 1.05)	1.00 (0.95, 1.06)	0.99 (0.94, 1.05)	1.00 (0.95, 1.05)
Conditional height 2-5 y	1.04 (0.99, 1.10)	1.02 (0.97, 1.08)	1.04 (0.99, 1.10)	1.02 (0.97, 1.08)
Conditional weight 0-2 y	1.03 (0.98, 1.08)	1.03 (0.99, 1.09)	1.03 (0.98, 1.08)	1.03 (0.98, 1.09)
Conditional weight 2-5 y	1.05 (1.00, 1.10)	1.04 (0.99, 1.09)	1.05 (1.00, 1.10)	1.04 (0.99, 1.09)
BMI trajectory[2]				
- 1	X	Ref	X	Ref
- 2	X	1.21 (0.95, 1.53)	X	1.19 (0.94, 1.51)
- 3	X	1.54 (1.18, 2.01)	X	1.54 (1.18, 2.00)
- 4	X	1.24 (1.01, 1.51)	X	1.26 (1.02, 1.54)
Pubic hair trajectory[3]				
- 1	X	X	Ref	Ref
- 2	X	X	1.10 (0.95, 1.26)	1.11 (0.96, 1.28)
- 3	X	X	1.04 (0.85, 1.28)	1.06 (0.87, 1.30)
Breast/genital development trajectory[3]				
- 1	X	X	Ref	Ref
- 2	X	X	0.94 (0.81, 1.10)	0.92 (0.78, 1.08)
- 3	X	X	0.96 (0.81, 1.14)	0.94 (0.79, 1.11)
- 4	X	X	1.03 (0.84, 1.25)	0.98 (0.80, 1.20)

[1] All models adjusted for sex, gestational age, maternal age, maternal years of education, parity, and age 0-2y physical asset score.

[2] BMI trajectory definitions: 1 – normal weight; 2 – early onset overweight to normal weight (males) or early onset obese to overweight (females); 3 – early onset overweight to obese (males) or early onset obese to morbidly obese (females); 4 – late onset overweight (females only)

[3] Trajectory 1 represents children who started puberty late and progresses slowly, with higher trajectories having progressively earlier pubertal start and faster tempo.

Table S5: Sex-specific adjusted associations of age 22 y allostatic load excluding 28 item General Health Questionnaire score with early life growth as potentially mediated by childhood/adolescent body mass index and adolescent pubertal trajectories in the Birth to Twenty Plus cohort (n = 596). Values displayed are adjusted risk ratios (95% CI) for a 1 unit increase in allostatic load measures estimated by Poisson regression.[1]

	Males (n = 282)				Females (n = 314)			
	Model 1	Model 2	Model 3	Model 4	Model 1	Model 2	Model 3	Model 4
Birthweight	1.06 (0.90, 1.24)	1.07 (0.91, 1.27)	1.06 (0.90, 1.25)	1.08 (0.92, 1.27)	1.03 (0.87, 1.23)	1.02 (0.86, 1.22)	1.05 (0.88, 1.25)	1.04 (0.87, 1.24)
Conditional height 0-2 y	0.98 (0.90, 1.05)	0.99 (0.91, 1.07)	0.98 (0.90, 1.06)	0.99 (0.91, 1.07)	1.04 (0.96, 1.12)	1.04 (0.96, 1.13)	1.02 (0.94, 1.10)	1.03 (0.95, 1.11)
Conditional height 2-5 y	1.06 (0.98, 1.14)	1.04 (0.96, 1.13)	1.05 (0.97, 1.14)	1.04 (0.96, 1.12)	1.09 (1.00, 1.18)	1.06 (0.97, 1.15)	1.08 (1.00, 1.17)	1.05 (0.97, 1.14)
Conditional weight 0-2 y	1.06 (0.99, 1.14)	1.06 (0.99, 1.14)	1.06 (0.99, 1.14)	1.06 (0.99, 1.14)	0.99 (0.92, 1.07)	1.00 (0.93, 1.08)	0.98 (0.91, 1.06)	1.00 (0.92, 1.08)
Conditional weight 2-5 y	1.08 (1.00, 1.17)	1.08 (1.00, 1.17)	1.08 (1.00, 1.17)	1.08 (0.99, 1.17)	1.02 (0.95, 1.09)	1.00 (0.93, 1.07)	1.01 (0.94, 1.08)	0.99 (0.92, 1.06)
BMI trajectory[2]								
- 1	X	Ref	X	Ref	X	Ref	X	Ref
- 2	X	1.21 (0.85, 1.71)	X	1.20 (0.84, 1.71)	X	1.25 (0.87, 1.79)	X	1.22 (0.85, 1.75)
- 3	X	1.76 (0.98, 3.17)	X	1.76 (0.97, 3.19)	X	1.64 (1.21, 2.24)	X	1.60 (1.17, 2.20)
- 4	-	-	-	-	X	1.32 (1.06, 1.63)	X	1.35 (1.08, 1.68)
Pubic hair trajectory[3]								
- 1	X	X	Ref	Ref	X	X	Ref	Ref
- 2	X	X	1.07 (0.82, 1.41)	1.06 (0.81, 1.39)	X	X	1.19 (0.99, 1.44)	1.22 (1.01, 1.47)
- 3	X	X	1.09 (0.76, 1.57)	1.09 (0.76, 1.56)	X	X	1.13 (0.86, 1.50)	1.17 (0.88, 1.54)
Breast/genital development trajectory[3]								
- 1	X	X	Ref	Ref	X	X	Ref	Ref
- 2	X	X	0.94 (0.71, 1.24)	0.94 (0.71, 1.23)	X	X	0.93 (0.74, 1.16)	0.88 (0.70, 1.11)
- 3	X	X	0.96 (0.70, 1.31)	0.94 (0.68, 1.28)	X	X	0.88 (0.70, 1.12)	0.86 (0.67, 1.09)
- 4	X	X	0.86 (0.58, 1.26)	0.85 (0.58, 1.26)	X	X	1.07 (0.83, 1.39)	1.00 (0.76, 1.30)

[1] All models adjusted for gestational age, maternal age, maternal years of education, parity, and age 0-2y physical asset score.

[2] BMI trajectory definitions: 1 – normal weight; 2 – early onset overweight to normal weight (males) or early onset obese to overweight (females); 3 – early onset overweight to obese (males) or early onset obese to morbidly obese (females); 4 – late onset overweight (females only)

[3] Trajectory 1 represents children who started puberty late and progresses slowly, with higher trajectories having progressively earlier pubertal start and faster tempo.

Table S6: Sex-specific unadjusted associations of high (> 4) age 22 y allostatic load with early life growth as potentially mediated by childhood/adolescent body mass index and adolescent pubertal trajectories in the Birth to Twenty Plus cohort (n = 596). Values displayed are unadjusted odds ratios (95% CI) estimated by logistic regression.

	Males (n = 282)				Females (n = 314)			
	Model 1	Model 2	Model 3	Model 4	Model 1	Model 2	Model 3	Model 4
Birthweight	1.01 (0.47, 2.20)	1.04 (0.47, 2.28)	0.99 (0.45, 2.18)	1.01 (0.45, 2.24)	1.23 (0.64, 2.35)	1.18 (0.61, 2.30)	1.22 (0.63, 2.38)	1.19 (0.61, 2.34)
Conditional height 0-2 y	0.90 (0.61, 1.33)	0.93 (0.63, 1.39)	0.89 (0.60, 1.33)	0.93 (0.62, 1.39)	1.11 (0.79, 1.55)	1.14 (0.80, 1.63)	1.06 (0.75, 1.49)	1.10 (0.76, 1.58)
Conditional height 2-5 y	1.16 (0.78, 1.72)	1.06 (0.70, 1.61)	1.15 (0.77, 1.72)	1.06 (0.69, 1.62)	1.38 (0.96, 1.98)	1.26 (0.86, 1.86)	1.36 (0.94, 1.98)	1.24 (0.84, 1.85)
Conditional weight 0-2 y	1.17 (0.83, 1.65)	1.14 (0.79, 1.62)	1.15 (0.81, 1.64)	1.13 (0.79, 1.62)	1.24 (0.89, 1.73)	1.31 (0.92, 1.85)	1.22 (0.87, 1.70)	1.29 (0.91, 1.83)
Conditional weight 2-5 y	1.66 (1.09, 2.52)	1.60 (1.04, 2.48)	1.66 (1.09, 2.55)	1.60 (1.03, 2.50)	1.00 (0.73, 1.36)	0.92 (0.67, 1.26)	0.96 (0.70, 1.33)	0.89 (0.64, 1.24)
BMI trajectory[1]								
- 1	X	Ref	X	Ref	X	Ref	X	Ref
- 2	X	2.54 (0.62, 10.40)	X	2.52 (0.59, 10.81)	X	2.71 (0.64, 11.45)	X	2.46 (0.58, 10.51)
- 3	X	6.23 (0.44, 87.23)	X	6.31 (0.43, 91.66)	X	4.86 (1.31, 18.08)	X	4.44 (1.15, 17.12)
- 4	-	-	-	-	X	2.58 (1.00, 6.62)	X	2.73 (1.04, 7.15)
Pubic hair trajectory[2]								
- 1	X	X	Ref	Ref	X	X	Ref	Ref
- 2	X	X	0.90 (0.22, 3.68)	0.83 (0.20, 3.39)	X	X	1.16 (0.49, 2.72)	1.27 (0.52, 3.07)
- 3	X	X	1.38 (0.25, 7.75)	1.28 (0.23, 7.05)	X	X	1.06 (0.32, 3.53)	1.18 (0.34, 4.15)
Breast/genital development trajectory[2]								
- 1	X	X	Ref	Ref	X	X	Ref	Ref
- 2	X	X	0.86 (0.21, 3.55)	0.87 (0.21, 3.58)	X	X	0.92 (0.32, 2.67)	0.78 (0.26, 2.34)
- 3	X	X	1.28 (0.26, 6.18)	1.21 (0.25, 5.83)	X	X	0.90 (0.30, 2.74)	0.82 (0.26, 2.57)
- 4	X	X	0.81 (0.12, 5.34)	0.81 (0.13, 5.13)	X	X	1.62 (0.51, 5.10)	1.26 (0.38, 4.21)

[1] BMI trajectory definitions: 1 – normal weight; 2 – early onset overweight to normal weight (males) or early onset obese to overweight (females); 3 – early onset overweight to obese (males) or early onset obese to morbidly obese (females); 4 – late onset overweight (females only)

[2] Trajectory 1 represents children who started puberty late and progresses slowly, with higher trajectories having progressively earlier pubertal start and faster tempo.

Table S7: Sex-specific adjusted associations of high (> 4) age 22 y allostatic load excluding 28 item General Health Questionnaire score with early life growth as potentially mediated by childhood/adolescent body mass index and adolescent pubertal trajectories in the Birth to Twenty Plus cohort (n = 596). Values displayed are adjusted odds ratios (95% CI) estimated by logistic regression.[1]

	Males (n = 282)				Females (n = 314)			
	Model 1	Model 2	Model 3	Model 4	Model 1	Model 2	Model 3	Model 4
Birthweight	1.19 (0.46, 3.10)	1.29 (0.49, 3.39)	1.13 (0.43, 3.01)	1.23 (0.46, 3.26)	0.96 (0.36, 2.56)	0.90 (0.33, 2.46)	1.09 (0.40, 2.93)	1.02 (0.37, 2.81)
Conditional height 0-2 y	0.92 (0.59, 1.42)	0.98 (0.62, 1.54)	0.91 (0.58, 1.41)	0.97 (0.61, 1.53)	1.10 (0.71, 1.70)	1.11 (0.70, 1.75)	1.04 (0.66, 1.63)	1.04 (0.65, 1.68)
Conditional height 2-5 y	1.36 (0.87, 2.13)	1.19 (0.74, 1.91)	1.36 (0.86, 2.15)	1.21 (0.75, 1.95)	1.64 (1.05, 2.55)	1.51 (0.94, 2.43)	1.68 (1.06, 2.65)	1.55 (0.95, 2.52)
Conditional weight 0-2 y	1.33 (0.89, 1.99)	1.32 (0.87, 2.00)	1.31 (0.87, 1.98)	1.31 (0.86, 2.01)	0.97 (0.63, 1.50)	1.00 (0.64, 1.56)	0.95 (0.62, 1.48)	0.99 (0.63, 1.55)
Conditional weight 2-5 y	1.88 (1.13, 3.13)	1.81 (1.05, 3.11)	1.90 (1.13, 3.19)	1.82 (1.05, 3.16)	1.14 (0.76, 1.72)	1.05 (0.69, 1.59)	1.09 (0.72, 1.65)	1.00 (0.65, 1.55)
BMI trajectory[1]								
- 1	X	Ref	X	Ref	X	Ref	X	Ref
- 2	X	3.20 (0.67, 15.30)	X	3.37 (0.69, 16.39)	X	2.32 (0.42, 12.75)	X	2.23 (0.39, 12.64)
- 3	X	10.32 (0.61, 174.62)	X	11.30 (0.63, 203.18)	X	4.31 (1.04, 17.83)	X	3.94 (0.90, 17.20)
- 4	-	-	-	-	X	2.22 (0.71, 6.94)	X	2.27 (0.71, 7.28)
Pubic hair trajectory[2]								
- 1	X	X	Ref	Ref	X	X	Ref	Ref
- 2	X	X	0.85 (0.17, 4.16)	0.74 (0.15, 3.68)	X	X	1.47 (0.48, 4.53)	1.61 (0.50, 5.13)
- 3	X	X	1.10 (0.15, 8.11)	0.99 (0.14, 7.09)	X	X	0.84 (0.17, 4.25)	0.91 (0.17, 4.90)
Breast/genital development trajectory[2]								
- 1	X	X	Ref	Ref	X	X	Ref	Ref
- 2	X	X	1.04 (0.21, 5.20)	1.03 (0.21, 5.17)	X	X	1.36 (0.31, 5.96)	1.21 (0.27, 5.53)
- 3	X	X	1.27 (0.20, 7.91)	1.17 (0.19, 7.34)	X	X	1.34 (0.29, 6.15)	1.21 (0.25, 5.80)
- 4	X	X	1.32 (0.16, 10.85)	1.40 (0.18, 10.71)	X	X	2.43 (0.49, 12.07)	1.92 (0.36, 10.26)

[1] All models adjusted for gestational age, maternal age, maternal years of education, parity, and age 0-2y physical asset score.

[2] BMI trajectory definitions: 1 – normal weight; 2 – early onset overweight to normal weight (males) or early onset obese to overweight (females); 3 – early onset overweight to obese (males) or early onset obese to morbidly obese (females); 4 – late onset overweight (females only)

[3] Trajectory 1 represents children who started puberty late and progresses slowly, with higher trajectories having progressively earlier pubertal start and faster tempo.

Table S8: Adjusted associations of high (> 4) age 22 y allostatic load with early life growth as potentially mediated by childhood/adolescent body mass index and adolescent pubertal trajectories in the Birth to Twenty Plus cohort (n = 596). Values displayed are adjusted odds ratios (95% CI) estimated by logistic regression.[1]

	Model 1	Model 2	Model 3	Model 4
Birthweight	1.11 (0.68, 1.82)	1.11 (0.67, 1.82)	1.09 (0.66, 1.80)	1.08 (0.65, 1.80)
Conditional height 0-2 y	1.00 (0.78, 1.28)	1.03 (0.80, 1.33)	0.98 (0.76, 1.26)	1.01 (0.78, 1.31)
Conditional height 2-5 y	1.23 (0.94, 1.60)	1.11 (0.84, 1.47)	1.21 (0.92, 1.58)	1.10 (0.83, 1.46)
Conditional weight 0-2 y	1.22 (0.96, 1.55)	1.23 (0.96, 1.57)	1.21 (0.96, 1.54)	1.23 (0.96, 1.57)
Conditional weight 2-5 y	1.23 (0.96, 1.57)	1.16 (0.90, 1.49)	1.21 (0.95, 1.56)	1.14 (0.88, 1.47)
BMI trajectory[2]				
- 1	X	Ref	X	Ref
- 2	X	2.73 (1.02, 7.28)	X	2.63 (0.98, 7.05)
- 3	X	5.13 (1.62, 16.22)	X	5.04 (1.56, 16.29)
- 4	X	2.22 (0.89, 5.54)	X	2.38 (0.94, 6.03)
Pubic hair trajectory[3]				
- 1	X	X	Ref	Ref
- 2	X	X	1.08 (0.53, 2.18)	1.13 (0.55, 2.33)
- 3	X	X	1.23 (0.47, 3.19)	1.32 (0.49, 3.53)
Breast/genital development trajectory[3]				
- 1	X	X	Ref	Ref
- 2	X	X	0.80 (0.36, 1.79)	0.71 (0.31, 1.62)
- 3	X	X	0.91 (0.39, 2.15)	0.81 (0.34, 1.95)
- 4	X	X	1.17 (0.46, 3.01)	0.95 (0.36, 2.51)

[1] All models adjusted for sex, gestational age, maternal age, maternal years of education, parity, and age 0-2y physical asset score.

[2] BMI trajectory definitions: 1 – normal weight; 2 – early onset overweight to normal weight (males) or early onset obese to overweight (females); 3 – early onset overweight to obese (males) or early onset obese to morbidly obese (females); 4 – late onset overweight (females only)

[3] Trajectory 1 represents children who started puberty late and progresses slowly, with higher trajectories having progressively earlier pubertal start and faster tempo.

3. Conclusions

3.1 Methodological considerations

3.1.1 Missing data

A substantial number of eligible participants in this analysis had missing data for at least one component of the AL score, with 276 participants missing data on four or more AL components and an additional 265 missing data on one to three AL components. In previous studies that calculated AL scores for participants, strategies for addressing missing data generally involved either utilizing complete cases (42, 45, 52) or using some form of simple imputation for participants missing a limited number of values (55, 59, 60). A complete case analysis, while straightforward to implement, will result in a loss of precision in the study estimates at best and, at worst, carries a risk of biasing the study results if the missing values are not missing completely at random. That is, if there is some reason other than chance why the missing values were not reported (i.e. participants with high BMI were less likely to consent to having their weight measured), simply dropping those participants can result in inaccurate model estimates. Simple imputation is also straightforward to implement but can result in artificially precise estimates by reducing the apparent variation in the data, as well as potentially introducing bias if the data are not missing completely at random. While most simple imputation approaches involve applying either the subject or sample mean to the missing item, Castagne et al (60) took the approach of assuming participants were not at risk for all missing items, which ensures that their estimates are, if anything, conservative.

For many of the studies that utilized one of these approaches, the percent of participants missing components of AL data was low, though the percent missingness was not reported in all cases. Complete case or simple imputation are unlikely to have a large effect if the amount of missingness is small, but in my analysis 26% of the eligible AL population was missing one to three AL measures. One strategy to accurately address and incorporate such levels of missingness is multiple imputation. In multiple imputation, each missing value is assigned an imputed value based on the observed values of other AL components and the model

covariates, along with a small amount of random error based on the size of the observed sample. This process is repeated to create several imputed datasets, all of which contain identical values for observed measures and slightly different values for imputed measures, reflecting the uncertainty in the imputed values. By running the specified analyses independently on each imputed dataset and pooling the model results according to the methods outlined by Rubin (71), the resulting model estimates are unbiased and have appropriately sized confidence intervals. I found only one example in the literature where the authors used this approach for their AL index. In Robertson et al's analysis, approximately 33% of their sample had incomplete AL information, which they imputed using multiple imputation (53).

With a non-trivial amount of missing values, as was the case in my analysis, using multiple imputation maximizes the available sample size while providing statistically correct point estimates and confidence intervals. For most analyses, modern computing power and software makes the process of creating and analysing imputed datasets a relatively simple process in the case where a substantial percentage of participants have missing data and such missingness cannot be assumed to be missing completely at random. Given this, increased use of multiple imputation or related techniques such as full information maximum likelihood estimation could help improve the precision and accuracy of research results.

In my analysis, I elected to exclude participants who were missing four or more components of the AL score, as those participants were generally missing an entire suite of components (i.e. all bloodwork-related measures) and the resulting imputed values would not have been able to be based on observed values of those theoretically related components. Including exclusions for missing the age 22 y visit, pregnancy or unknown pregnancy status, and CRP values that indicated an acute infection, I excluded 2237 participants. Only 512 of those excluded attended the age 22 y visit, with 276 excluded due to missing four or more AL components.

Comparisons of excluded with included participants revealed that those who were excluded were more likely to be born at a later gestational age, have a higher household asset score early in life, and be of a non-Black ethnicity (Supplemental Table S1). The ethnicity differences are expected as recruiting for later Bt20+ waves focused on Black participants. Differences in gestational age and early life asset

score, while statistically significant, were quite small in practical terms. The overall similarity between the included and excluded group suggests that the exclusions used were unlikely to severely bias the results and suggests that the results are likely generalisable to similar urban South African populations.

3.1.2 Differing allostatic load calculation methods

A key issue identified in reviews of AL methods is the variety of methodologies used in calculating the AL score (43, 51). While the variety of physiological components that make up an AL score in the literature remains an issue, this is largely driven by the availability of data in the study population. Calculations of AL are largely conducted *post-hoc* after the completion of data collection, making the methodological choice of how to calculate AL the one that remains fully under the control of the researcher. A 'gold standard' of AL measurement and calculation has yet to be defined in the literature. For this analysis, I used the predominant trend in the literature, which is a summary index of indicators for which an individual's values are in the top empirical quartile.

While some studies have made use of more complex measures to calculate AL (61, 72), early studies that compared the simple, count-based measure to alternative methods found little difference in predictive value of AL (42, 54). Since that point, however, there is little to no evidence directly comparing different AL calculations methods, such as factor analysis, canonical correlations, or summative indexes, to each other. Though beyond the scope of this dissertation, an overarching analysis comparing predictive properties of different AL calculation methods for future health using the same underlying data would be beneficial to assist study design for future researchers.

AL is related in concept to the idea of a metabolic syndrome (MS) score, with similar discussions regarding the correct way to operationalise the concept. Metabolic syndrome traditionally encompasses a narrower set of risk factors than AL such as central obesity, blood pressure, cholesterol, and fasting glucose, with elevated levels of at least three indicators considered a marker of metabolic syndrome (73). Recent years have seen publications advocating for the use of a continuous MS score rather than the original indicator to help better predict later health outcomes (74, 75). While the International Diabetes Foundation issued a

consensus statement in 2007 regarding the measurement of MS in youth (76), there remains some debate in the literature regarding which risk factors should be included in the MS evaluation and at what ages MS can be diagnosed (77).

Methodological discussions around MS generally focus around specific calculation methodology rather than on heterogeneity in included components, which dominate discussions of AL operationalisations (43, 51). There has not yet been an effort like that of the International Diabetes Foundation to create a standardised definition of AL, with the included components varying widely from one study to the next. Creation of such a standardised definition would help harmonise definitions of AL across future studies and facilitate future comparisons of results between studies.

3.2 Broader implications and further research

3.2.1 Identifying young adults at risk

While early life growth was not associated with AL in young adulthood, I did find associations between high childhood and adolescent BMI and increased AL. These associations were present overall when controlling for sex and were significant in females in sex-specific analyses, though the small number of males in the higher BMI trajectories makes it difficult to draw conclusions for that group. These associations were present when analysing AL both as a count measure (Table 2.3 and Supplemental Table S4), and as a binary high-risk measure (Table 2.4 and Supplemental Table S8). No matter how AL was analysed, being a member of the ‘early onset overweight/obese to obese/morbidly obese’ BMI trajectory during childhood and adolescence was strongly associated with a higher AL score. These associations were not as strong or consistent for member of the ‘overweight/obese to normal weight/overweight’ trajectory, suggesting that decreases in adiposity later in adolescence can have positive impacts on young adult health. This study provides further empirical evidence that continuing increased adiposity during the childhood and adolescent years is associated with adverse health outcomes in young adulthood. In the current study, such an association is clearly indicated for young women and, though pooled results suggest an overall relationship, conclusions for young men should be interpreted with caution due to the small number of males in the high BMI trajectories.

3.2.2 Clarifying links to older adult health outcomes

Much of the research on links between AL score and later adverse health outcomes has been conducted in older (70+ y) adults (42, 54), or focused on associations between AL in middle age and resulting mortality (53, 60). By using data from the Bt20+ cohort, which remains an active cohort, this research lays the groundwork for future work to examine associations between AL in early adulthood and adverse health outcomes throughout adult life.

A key question to examine will be whether AL score as a continuous measure predicts adult health outcomes, or whether there is a critical cut-off for AL score above which adverse health outcomes begin to appear. Seeman et al's studies of the MacArthur studies of successful aging used a continuous measure of AL as a predictor of future health outcomes (42, 54), as did Robertson et al's analysis of mortality in the Scottish Health Survey (53), while Castagne et al's analysis of mortality using the 1958 British birth cohort used tertiles of AL score (60). Both Robertson et al and Castagne et al's analyses showed significant associations between higher AL score and risk of mortality, indicating that while a high AL cut-off is useful to determine later risk of mortality there may be some 'dose-response' effect between the level of AL score and subsequent risk of mortality. As a longitudinal birth cohort, Birth to Twenty Plus is uniquely positioned to collect data on both mortality and morbidity in the coming years and shed light on not only whether AL in early adulthood is associated with later health, but whether that relationship follows an incremental relationship with increasing AL score or is only apparent once AL reaches a critical high-risk cut-off value.

3.2.3 Sex differences in contributors to allostatic load

An interesting result in my research was the finding that, while the overall AL score was similar between males and females, the components that contributed to the AL score varied widely by sex. Sex differences in the prevalence of many components of AL are well documented, but most previous operationalisations of AL used an overall, non-sex-specific, empirical cut point for high risk status, analogous to the methodology I used. As AL is meant to be a measure of overall physiological risk, such an approach essentially states that having a high-risk value for any contributor to AL is equally detrimental to overall health. Previous research on the

predictive value of AL has found that the overall score can be a better predictor of later health than individual components (42), but that research was conducted in older (age 70+ y) adults. As more research is done to link early adulthood AL to later health outcomes, it is possible that sex-specific components that have a greater impact on any association between AL and later adverse health outcomes will emerge through sex-specific analyses.

3.3 Concluding points

Using longitudinal data from the Birth to Twenty Plus cohort, I found no association between early life height and overall physiological health in early adulthood as measured by an allostatic load score, using multiple imputation to take advantage of partial information available. Increasing adiposity during childhood and adolescence increases the risk of higher allostatic load at age 22 y, particularly among females. Further research is needed to expound potential links between early adulthood allostatic load and later adverse health outcomes as well as provide clarity on the utility of different methodologies to calculate allostatic load. In this urban population, the greatest risk to young adult health is gaining too much weight during childhood, which results in a significant prevalence of overweight and obesity, particularly among the girls. The value of either a composite or single-item score is to assist public health efforts to identify health burden priorities and strategies. This work highlights that a pertinent area to invest interventions into is prevention efforts to minimise excessive weight gain in childhood.

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Appendices:

Appendix A: Ethics clearance 52

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

R14/49 Mr Craig McGowan

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180933

NAME: (Principal Investigator)	Mr Craig McGowan
DEPARTMENT:	School of Clinical Medicine Paediatrics Developmental Pathways for Health Research Unit Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Associations of early life growth with health at age 22 years as measured by an allostatic load index: Birth to Twenty Plus Cohort
DATE CONSIDERED:	28/09/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Shane Norris
APPROVED BY:	CBPenny Dr C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	05/10/2018

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 Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. 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**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed September and will therefore be due in the month of September each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES