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Wibæk Christensen, Rasmus; Girma, Tsinuel; Admassu Wossen, Bitiya; Abera Mengistie, Mubarek; Abdissa, Alemseged; Geto, Zeleke; Kæstel, Pernille; Vistisen, Dorte; Jørgensen, Marit Eika; Wells, Jonathan C K; Michaelsen, Kim F.; Friis, Henrik; Andersen, Gregers Stig

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Title

Higher weight and weight gain after 4 years of age rather than weight at birth are associated with adiposity, markers of glucose metabolism and blood pressure in 5-year-old Ethiopian Children

Author names

Rasmus Wibaek[1,2], Tsinuel Girma[3,4], Bitiya Admassu[1,4,5], Mubarek Abera[1,4,6], Alemseged Abdissa[4,7], Zeleke Geto[8], Pernille Kæstel[1], Dorte Vistisen[2], Marit E Jørgensen[2,10], Jonathan CK Wells[9], Kim F Michaelsen[1], Henrik Friis[1], Gregers S Andersen[2]

Author affiliations

[1]Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark; [2]Clinical Epidemiology, Steno Diabetes Center Copenhagen, Denmark; [3]Department of Pediatrics and Child Health, Jimma University, Ethiopia; [4]JUCAN Research Center, Jimma University, Ethiopia; [5]Department of Population and Family Health, Jimma University, Ethiopia; [6]Department of Psychiatry, Jimma University, Ethiopia; [7]Department of Laboratory Sciences and Pathology, Jimma University; [8]Ethiopian Public Health Institute, Ethiopia; [9]Childhood Nutrition Research Centre, UCL Great Ormond Street Institute of Child Health, United Kingdom; [10]National Institute of Public Health, Southern Denmark University, Denmark.

Corresponding Author

Rasmus Wibaek, Clinical Epidemiology, Steno Diabetes Center Copenhagen, Niels Steensens Vej 2, 2820 Gentofte, Denmark; Telephone: +45 28302381; E-mail: rwibaek@gmail.com.

Authors' last names

Wibaek, Girma, Admassu, Abera, Abdissa, Geto, Kæstel, Vistisen, Jørgensen, Wells, Michaelsen, Friis, Andersen.

Short running head

Weight gain and cardiometabolic markers at 5 years

Supplementary data

Supplemental Tables 1-2, Supplemental Figures 1-9, Supplemental Methods and Supplemental References are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.

Abbreviations

Body composition (BC), confidence interval (CI), developmental origins of health and disease (DOHaD), fat mass (FM), fat-free mass (FFM), homeostasis model assessment of insulin resistance index (HOMA-IR), infant Anthropometry and Body Composition (iABC), International Wealth Index (IWI), linear-spline mixed effects (LSME), standard deviation score (SDs), World Health Organization (WHO).

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Conflict of Interest (COI) Statement

None of the authors had any conflicts of interest.

Abstract

Background

Fetal and early life growth is associated with adult risk of obesity and cardiometabolic disease. However, little is known about the relative importance of birth weight and successive periods of weight gain on markers of cardiometabolic risk in childhood in low-income populations.

Objective

The objective was to study associations of birth weight and weight gain velocities in selected age intervals from birth to 60 mo with height, fat-free mass and markers of adiposity and cardiometabolic risk at 60 mo.

Design

In a prospective cohort study of 375 Ethiopian children aged 60 mo, we estimated individual weight gain velocities in the periods between birth, 3, 6, 24, 48 and 60 mo using linear-spline mixed- effects modelling. Subsequently, we analyzed associations of birth weight, weight gain velocities and current weight with height, fat-free mass and markers of adiposity and cardiometabolic risk.

Results

Weight gain from 48-60 mo and weight at 60 mo rather than birth weight were the strongest correlates of insulin, C-peptide, HOMA-IR, blood pressure, height, fat-free mass, waist circumference and fat mass at 60 mo. For instance, 1 SDs higher (1 SDs = 50 g/mo) weight accretion from 48-60 mo was associated with a higher insulin of 23.3% (95% confidence interval: 9.6, 38.8), C-peptide of 11.4% (2.7, 20.8), systolic blood pressure of 1.4 mmHg (0.6,

2.3), fat mass of 0.72 kg (0.59, 0.85), and fat-free mass of 0.70 kg (0.56, 0.85). Weight gain from 0-3 mo was positively associated with LDL cholesterol, systolic blood pressure, height and the BC indices, and weight gain from 24-48 mo was inversely associated with blood glucose.

Conclusions

In 60-mo-old Ethiopian urban children, weight gain and weight after 48 mo rather than weight at birth may represent a sensitive period for variations in markers of adiposity and glucose metabolism.

Clinical Trial Registry

The birth cohort is registered in ISRCTN (https://www.isrctn.com/): identifier ISRCTN46718296.

Keywords

birth weight; body composition; cardiometabolic status; cohort study; child, preschool; developmental origins of health and disease; Ethiopia; growth; infant; linear-spline mixed-effects model.

Introduction

Growth patterns in fetal life and early childhood have been associated with risk of obesity and cardiometabolic disease in adulthood in numerous studies (1-5), and it has been suggested that the risk of detrimental cardiometabolic adaptations is most pronounced when poor pre- and postnatal growth are followed by accelerated weight or body mass index (BMI) gain in childhood (6-10). This is commonly referred to as the developmental origins of health and disease (DOHaD) hypothesis, where environmental stimuli or insults in fetal life and early childhood, can cause lasting metabolic alterations in the developing child. Studies from middle- (11-13) and high-income populations (14-23) have found that cardiometabolic adaptations related to accelerated growth in early life may be initiated already in childhood. Thus far, no studies from low-income countries have examined the relative importance of birth weight and successive periods of weight gain in early life on subsequent body composition (BC) and markers of cardiometabolic risk in early childhood. This is surprising, as the combination of persistent high rates of undernutrition, steadily increasing rates of childhood and adult obesity, and an accelerating nutritional transition are likely to make these populations particularly vulnerable to the programming effects of growth in early life (24-26). Furthermore, the effects of variability in early-life growth on later BC appear to be population-specific (27, 28). Thus, to develop appropriate nutrition-specific interventions to prevent the emerging epidemic of obesity and cardiometabolic disease facing many low-income countries in sub-Saharan Africa (29, 30), it is important to improve our understanding of the timing of how growth in early-life is related to markers of adiposity and cardiometabolic risk in these settings.

Commonly, studies in the DOHaD literature have assessed growth from a single or only a few measurements such as fetal growth indexed by birth weight or weight change from birth to two years. With these study designs it is not possible to examine the dynamic weight changes that occur throughout infancy. Moreover, life-course studies of fetal and early childhood antecedents of later disease risk often rely on unbalanced data, where repeated measures within individuals are not measured at exactly the same time points and the sample size at each time point are changing. Therefore, appropriate analyses require statistical methods that can flexibly and robustly estimate dynamic changes in early life and are able to account for the dependencies of repeated measures within the same individual as well as an unbalanced data structure (31, 32). We modelled changes in weight in early childhood using Linear-spline Mixed-effects (LSME) modelling which can accommodate these issues. In this longitudinal study, we therefore aimed to investigate the associations of birth weight, weight gain velocities in selected age intervals from birth to 60 mo and weight at 60 mo with BC and markers of cardiometabolic status at 60 mo of age in Ethiopian children.

Subjects and methods

Study setting and participants

Infants and mothers were enrolled in the infant Anthropometry and Body Composition (iABC) birth cohort study between December 2008 and October 2012 at Jimma University Specialized Hospital, Jimma, Ethiopia. All mothers residing in the town of Jimma (estimated population of 157,432 (33)), giving birth to a term child (gestation >37 weeks) with a birth weight of ≥1500 g without congenital malformations were eligible to participate. If informed consent was obtained, the children were enrolled and examined within 48 hours after delivery. The study participants were scheduled for 12 visits between birth and 60 mo of age (0, 1.5, 2.5, 3.5, 4.5, 6, 12, 18, 24, 36, 48, 60 mo). A detailed description of the iABC cohort is found elsewhere (34, 35).

Data collection

Anthropometry from birth to 60 mo

Weight from birth to 6 mo was measured to the nearest 0.1 g using the inbuilt scale of a PEA POD (COSMED, Rome, Italy), from 12-36 mo to the nearest 0.1 kg using an electronic UNICEF scale (SECA, Hamburg, Germany) and from 48-60 mo to the nearest 1 g using the inbuilt scale of a BOD POD (COSMED, Rome, Italy). Height at 60 mo was measured to the nearest 0.1 cm using a SECA 213 portable height measurer (SECA, Hamburg, Germany). Waist circumference at 60 mo was measured to the nearest 0.1 cm in standing position with feet together midway between the iliac crest and lowest costal margin using a non-stretchable measuring tape. The anthropometric measurements were done by two research nurses, trained according to the standard operating procedures as recommended by the World

Health Organization (WHO) (36). Throughout the data collection period, we ran regular refreshment trainings as well as interobserver monitoring of the anthropometry assessments to ensure accuracy and reliability.

Body composition at 60 mo

Fat mass (FM) and fat-free mass (FFM) at 60 mo were measured using the BOD POD – an air displacement plethysmograph with a pediatric chair insert. The BOD POD system is an accurate, precise, feasible and safe method for assessment of BC in children (37, 38). During the measurement, the child was placed in a pediatric chair insert in an enclosed test chamber, not wearing any clothes besides a swim cap and tight fitted underpants. A complete BOD POD measurement lasted approximately 5-10 minutes. Each morning the research nurses calibrated both the PEA POD and the BOD POD using standardized weight and volume cylinders from the manufacturer to ensure accurate and precise assessments of child weight and volume. All the calculations were performed by the inbuilt computer of the BOD POD (software version 5.2.0). A detailed description of the theory and calculations behind the BC method can be found elsewhere (39).

Blood pressure at 60 mo

Systolic and diastolic blood pressure (mmHg) were measured in sitting position after the child had relaxed for a minimum of 5 minutes. Measurement were done in duplicate using a blood pressure monitor with age-appropriate cuffs and averaged (Pressostabil model, Welch Allyn Inc., Skaneateles Falls, USA).

Cardiometabolic markers at 60 mo

A 2 mL venous blood sample was collected from the antecubital fossa by a laboratory technician. The mother was instructed not to give her child any food or drinks 2 hours prior to arriving at the clinic, and the blood was sampled as the last element in the assessment battery, yielding a minimum of 3 hours of fasting. Blood glucose (mmol/L) was determined on whole blood samples using the HemoCue Glucose System (HemoCue, Ängelholm, Sweden). Glycosylated hemoglobin (HbA1c, mmol/mol) was measured on whole blood samples using a DCCT aligned Quo-Test® A1c Analyzer (EKF Diagnostics, Cardiff, Wales). Subsequently, serum was obtained by centrifuging the whole blood sample, aliquoted in 3x0.4 mL and frozen at -80°C until analyzed. The serum samples were analyzed at the Ethiopian Public Health Institute, using the module c501 of the COBAS 6000 analyzer (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) for total-, LDL- and HDL cholesterol and triglyceride concentrations (all lipids in mmol/L) and the module e601 for insulin (µU/mL) and C-peptide (ng/mL). The homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated as insulin × glucose / 22.5.

Covariates

Information on birth order, sex, gestational age, maternal age and educational level, family socioeconomic status and breastfeeding status was collected through questionnaires, that were double entered and corrected for any discrepancies. Maternal height was measured to the nearest 0.1 cm using a Seca 214 Stadiometer (SECA, Hamburg, Germany). Gestational age was obtained by physical examination of the newborn by trained research nurses using the New Ballard Score test (40). Family socioeconomic status was estimated using the International Wealth Index (IWI), a comprehensively tested index of the material well-being

of households in low and middle income countries (41, 42). IWI uses information of 12 material well-being dimensions, including 7 household assets, access to 2 public services, and 3 characteristics of the house to measure the extent to which the basic needs of the household are met. IWI is measured on a scale from 0 to 100 (highest wealth). Breastfeeding status at 4 to 6 mo post-partum was divided into 4 categories: exclusive (no other foods given), almost exclusive (no other foods given except water), predominant (breast milk as primary food) and partial/none (breast milk not the primary food/not breast feeding) (43). These covariates were considered potential confounding variables in the linear regression models.

Ethics

Ethical permission was granted from the Jimma University Ethical Review Committee (Ref. no. RPGC/279/2013). Written informed consent was obtained from parents or caregivers of all eligible participants. There were no risks related to the examinations, and before the 2 ml of venous blood was drawn from the child a topical anesthetic (EMLA crème) was applied to the skin. Children with medical conditions observed by the research nurses during the examinations were referred according to local clinical guidelines.

Statistical methods

Individual weight gain velocities were computed for 5 selected age intervals from birth to 60 mo of age (Step 1), and then associations of birth weight, weight gain velocities and current weight with BC and cardiometabolic markers at 60 mo were modelled (Step 2).

Estimated birth weight and weight gain velocities from 0-60 mo

To approximate the non-linear relationship between weight and age in children, a linear-spline mixed effects (LSME) model was applied to estimate the individual weight gain velocities in 5 pre-specified age intervals from birth to 60 mo (44, 45). The LSME model builds upon a mixed effects modelling approach by joining together two or more linear mixed effects functions at the knot points of pre-specified age intervals. Within each age interval the growth velocity or the slope of the curve is specified to be linear. This allows children to change growth velocity as they age from birth to 60 mo. Children with at least one weight measurement at birth and at the 60-mo visit were included in the modelling of the individual growth velocities. A more technical description of the LSME modelling for this study is presented in the Supplementary data **(Supplemental Methods)**.

Associations of birth weight and weight gain velocities with body composition and cardiometabolic markers

In separate models, the cardiometabolic markers measured at the 60-mo visit were regressed on estimated birth weight and the weight gain velocities derived in step 1 as well as on current weight at the 60-mo visit, respectively. Prior to the regression analysis, the birth weight, weight gain velocities and current weight were standardized to a standard deviation score (SDs) of 1 and a mean of 0 to obtain comparable model estimates across the different growth periods. Thus, the beta coefficients from the regression analyses indicate the change in outcome per study population SDs of the exposure variables (e.g. weight gain from 0-3 mo). The analyses were adjusted for the following covariates in separate models: Model 1 was adjusted for sex, birth order and gestational age. Model 2 was additionally adjusted for the child's exact age at the 60-mo visit, maternal age at delivery, maternal

postpartum height, maternal educational status and family socioeconomic status (IWI). Model 3, which was considered the main model of this study, was additionally adjusted for child birth weight. To assess whether potential associations observed in model 3 were mediated by weight at 60 mo, a model 4 was additionally adjusted for weight at the 60-mo visit. In the analyses of the outcomes systolic and diastolic blood pressure model 4 was adjusted for height and weight at 60 mo in addition to the adjustments in model 3. The analyses of estimated birth weight as primary predictor did not include a model 3, and the model 4 in these analyses was in addition to the adjustments in model 2 adjusted for child weight at 60 mo. The analyses of weight at 60 mo as primary predictor did not include a model 4 for all outcomes except for systolic and diastolic blood pressure, where model 4 was adjusted for height at 60 mo in addition to the adjustments in model 3. To compare the estimated associations across model 1–4 for a given outcome and exposure, we used a complete case approach, limiting the analyses to data with complete information on all covariates in model 4. In addition to the main analyses, we ran a number of sensitivity analyses. First, information on breastfeeding status was available on a smaller subsample. Thus, in a sensitivity analysis, we adjusted model 1-4 in all analyses for breastfeeding status at 4 to 6 mo post-partum to assess the potential change of estimates. Second, we restricted the analyses to only term average for gestational age children (i.e. children born at term with at birth weight >2500 g) to assess how the exclusion of small for gestational age children affected the results. Third, the standard error of the estimated weight gain velocity trajectories for children contributing with fewer weight measurements from 0-60 mo to the modelling are expected to be higher than for children with more weight measurement from 0-60 mo. Therefore, in an additional sensitivity analysis, we adjusted for the uncertainty in

the estimations of the child-specific weight gain velocities (i.e. estimated standard-errors of the child-specific random effects). This was done for model 1-4 in all analyses, except for the analyses where the observed weight at 60 mo was the primary predictor. Lastly, in a final sensitivity analysis, we accounted all analyses in model 3 (the main model) for multiple testing using the Benjamini-Hochberg approach (46).

All descriptive data are presented as mean (SD) or median (interquartile range) for continuous variables and percentages for categorical variables. Outcome variables found not to follow a normal distribution (i.e. insulin, C-peptide, HOMA-IR and triglycerides) were log-transformed prior to the regression analyses. The resulting effect estimates were back-transformed and presented as percentwise change. P values <0.05 were considered statistically significant. The LSME modelling was carried out using the “lmer” function in the “lme4” package in R. All analyses were carried out in R version 3.4.1 (The R foundation for Statistical Computing).

Results

A flow diagram of the study participants is shown in **Figure 1**. A total of 375 children were included in the LSME modelling of weight gain from birth to 60 mo (step 1), and 367 children had full covariate information for the subsequent regression analyses (step 2). A description of the study population is shown in **Table 1**. At birth, 10% were low birth weight, and according to international growth standards (WHO) (47), at 60 mo, the children were on average slightly thinner, lighter and shorter, and 7% were overweight or obese. The prevalence of stunting recorded in this population was similar to that of the capital Addis Ababa (15%), but lower than the national urban average of 25% (48). The average socioeconomic status of the study population (IWI score: 46 out of 100) was considerably higher than rural Ethiopia (12 out of 100), and slightly less than the urban areas of the country (52 out of 100) (41, 42). Boys and girls were equally distributed in the study sample, and at birth boys had slightly more FFM than girls. The average age at the 60-mo visit was 59.95 (SDs 1.47) mo. For the subsequent regression analyses (step 2), the total number of children included in the analyses differed depending on the specific outcome, as some participants had refused the blood sampling or had only been able to deliver an amount of blood that was insufficient for all biomarkers to be analyzed **(Table 2)**.

Child growth from 0-60 mo

The children had their weight measured a median (interquartile range) of 9 (8-10) times from birth to 60 mo and contributed with a total of 3336 weight measurements to the growth modelling. The distribution of the weight measurements at each follow-up visit is

shown in Figure 1. A model with knot points placed at 3, 6, 24 and 48 mo yielded the lowest Bayesian information criterion value and was selected as the best fitting model. Thus, the model estimated 6 parameters representing the estimated individual birth weight and 5 weight gain velocities from birth to 60 mo, and thus the deviance from the average birth weight and weight gain velocities in the periods 0-3, 3-6, 6-24, 24-48 and 48-60 mo. On average, the weight gain velocities declined gradually over the 5 growth periods from 1,012 g/mo from 0-3 mo to 139 g/mo from 48-60 mo (**Table 3**). The variation in the weight gain velocities also declined steadily from 0-3 mo to 24-60 mo (**Supplemental Figure 1**). Compared to the international growth standards (WHO), boys and girls were on average more than 200 g lighter at birth but gained weight at a higher velocity from 0-6 mo. However, from 6 to 60 mo they gained weight at a lower velocity, resulting in a considerable average weight deficit of 1,915 g for girls and 1,885 g for boys at 60 mo compared with the WHO reference. The median growth velocity curve for the study population estimated from LSME modelling compared with the weight-for-age growth reference is shown in **Figure 2**. Furthermore, the model fits for 3 example children with different growth trajectories is shown in **Supplemental Figure 2** and the individual model fits for all included children are shown in **Supplemental Figure 3**. Correlations of varying strengths were observed between the estimated birth weight and weight gain velocities **(Supplemental Figure 4)**. A matrix of the model assumptions tests of the LSME modelling is shown in **Supplemental Figure 5.**

Birth weight, child growth and cardiometabolic markers at age 5

Associations of estimated birth weight, weight gain velocities and observed weight at 60 mo with BC and cardiometabolic markers are presented in **Figure 3** and **Supplemental Table 1**. Independent of birth weight and the other covariates (model 3), higher weight accretion from 48-60 mo was associated with higher levels of insulin, C-peptide, HOMA-IR and HDL cholesterol at 60-mo. For instance, 1 SDs higher (1 SDs = 50 g/mo) weight accretion from 48-60 mo was associated with a higher HOMA-IR of 24.3% (95% confidence interval (CI): 9.6, 41.0), insulin of 23.3% (9.6, 38.8), C-peptide of 11.4% (2.7, 20.8) and HDL cholesterol of 0.03 mmol/L (0.00, 0.06). The associations for insulin, C-peptide and HOMA-IR remained after adjusting for current weight (model 4). Additionally, higher weight at 60 mo was associated with higher insulin and HOMA-IR. Higher weight accretion from 24-48 mo (1 SDs = 38 g/mo) was associated with lower levels of blood glucose levels at 60 mo [$\beta_{model\ 3}$ = -0.11 mmol/L (95% CI: -0.21, -0.02)]. Higher weight accretion from 0-3 mo (1 SDs = 165 g/mo) was associated with higher levels of LDL cholesterol [$\beta_{model\ 3}$ = 0.08 mmol/L (0.01, 0.16)]. The associations for blood glucose and LDL cholesterol remained after adjusting for current weight. Additionally, in model 4, higher weight accretion from 6-24 mo (1 SDs = 49 g/mo) was associated with lower levels of LDL cholesterol [$\beta_{model\ 4}$ = -0.09 mmol/L (-0.16, -0.01)]. We found no associations of weight at birth, weight at 60 mo and weight accretion in any of the age periods with HbA1c, total cholesterol and triglycerides. Regarding blood pressure at 60 mo, in model 3, positive associations were seen with weight at 60 mo and weight accretion in the age periods from 0-60 mo (systolic) and 24-60 mo (diastolic). For instance, 1 SDs higher (1 SDs = 2,161 g) weight at 60 mo was associated with a higher systolic blood pressure of 2.5 mmHg (1.6, 3.3). The positive associations seen for systolic and diastolic

blood pressure and weight accretion in the age periods from 0-60 mo disappeared when adjusting for current weight and height at 60 mo (model 4). However, in model 4, the positive association of weight 60 mo with systolic blood pressure remained, while negative association of weight at birth with systolic blood pressure appeared. Birth weight, weight accretion in the age periods from 0-60 mo and weight at 60 mo were positively associated with height, waist-circumference, FM and FFM at 60 mo. For instance, in model 3, 1 SDs higher (1 SDs = 50 g/mo) weight accretion from 48-60 mo was associated with a greater height of 1.7 cm (95% CI: 1.2, 2.1), waist circumference of 1.6 cm (1.3, 2.0), FM of 0.72 kg (0.59, 0.85) and FFM of 0.70 kg (0.56, 0.85). The associations largely attenuated after adjusting for current weight (model 4).

The additional sensitivity analyses adjusting the analyses for breastfeeding status at 4-6-mo post-partum **(Supplemental Figure 6)**, restricting the analyses to average for gestational age children **(Supplemental Figure 7)** and adjusting the analyses for uncertainty of the child-specific weight gain velocity estimations **(Supplemental Figure 8)** did not alter the presented associations noticeably. Furthermore, when accounting for multiple testing of the results presented in model 3 (the main model), the associations of weight accretion from 48-60 mo with insulin, C-peptide, HOMA-IR and blood pressure as well as most of the other associations for blood pressure and all associations for height, waist-circumference, FM and FFM remained significant despite the lower alpha-level in the Benjamini-Hochberg approach **(Supplemental Figure 9)**. However, the associations for blood glucose, HDL- and LDL cholesterol lost significance when accounting for multiple testing.

Discussion

In this study, we present the first results from a low-income country on the effects of weight gain velocity in a number of critical windows in early life on linear growth, FFM and markers of adiposity and cardiometabolic risk in childhood. Independent of birth weight and the included covariates, weight gain from 48-60 mo was positively associated with insulin, C-peptide, HOMA-IR, HDL cholesterol, blood pressure, height, waist circumference, FM and FFM at 60 mo. Moreover, weight gain from 0-3 mo was positively associated with LDL cholesterol, systolic blood pressure, height, waist circumference, FM and FFM at 60 mo, while weight gain from 24-48 mo was inversely associated with blood glucose. Birth weight, on the other hand, was only positively associated with height, waist circumference, FM and FFM at 60 mo.

Few studies from middle- and high-income countries have investigated the relative importance of birth weight and successive periods of growth in early life on markers of cardiometabolic risk in early childhood, and evidence from sub-Saharan Africa is limited to South African children. Crowther et al. showed that higher weight gain from birth to 4 years and beyond, but not between birth and 1 year, was associated with higher insulin levels in South African children (13). In a study of Chilean children, Corvalán et al. found that BMI growth from 1.5-4 years presented the strongest association with a composite metabolic risk score, but did not find growth from birth to 4 years to be associated with HOMA-IR or plasma lipids (18). Joglekar et al., on the other hand, found that higher weight gain in selected periods after 6 mo of age, was associated with systolic blood pressure and HOMA-IR at 6 years in children from rural India, and that associations were stronger for the most recent growth periods (11). Similarly, in 6-year-old Dutch children, Voerman et al. found that

conditional weight gain from 4-6 years were more strongly associated with insulin and C-peptide levels at 6 years than earlier periods of weight gain (15). These findings are consistent with the present study in suggesting that growth in early childhood rather than in infancy and fetal life is the strongest correlate of levels of C-peptide, insulin and insulin resistance (HOMA-IR). However, it is difficult to say if increased levels of the studied cardiometabolic markers are harmful for the children in the longer term or rather represent normal and beneficial metabolic adaptations to increased weight gain.

Birth weight and weight gain velocity in all age periods as well as weight at 60 mo was positively associated with height, waist circumference, FM and FFM. The associations were strongest for weight at 60 mo and the most recent growth periods showed larger effect estimates. Interestingly, the effect sizes for FM were very similar to those of FFM despite FFM at 60 mo on average was an almost 3 times larger body compartment than FM. In Indian children, Joglekar et al. found that weight gain from birth to 6 years was associated with both FM and FFM, but with stronger associations for FFM (11). In Dutch children, de Beer et al. found that weight gain from 0-12 mo showed a stronger association with FM compared to FFM at 5-6 years (22). Our analyses suggest that weight gain from birth to 60 mo, is being allocated in relatively larger amounts to FM storage compared to FFM, irrespectively of weight status at birth. Compared to BC references derived in UK children, our participants had higher FM (boys: 3.11 vs. 4.19 kg, and girls: 3.97 vs. 4.14 kg) and markedly lower FFM (boys: 16.35 vs. 12.27 kg, and girls: 14.60 vs. 12.04 kg) at 60 mo (49). Thus, despite being born with an average deficit in weight that persisted at 60 mo of age when comparing to the international growth standards (WHO), these children underwent a significant catch-up in FM associated with weight changes in both infancy and early

childhood and resulting in a considerably higher FM at 60 mo compared to children from a high-income country.

The mechanisms that explain the associations of variability in early growth with markers of adiposity and cardiometabolic risk in childhood remains unclear, but they may include alterations to the microbiome, hepatic endoplasmic reticulum stress and MicroRNAs associated with impaired insulin signaling, as well as epigenetic and transcriptional mechanisms (50). The epigenetic mechanisms include DNA methylation and histone modifications that induce changes in the structure and function of vital organs related the metabolic homeostasis such as the liver, adipose tissue and pancreas (50-52). Ibáñez et al. found that catch-up in weight from birth to 4 years in children small-for-gestational age elevated adiposity and promoted insulin resistance (14). Moreover, as proposed in the capacity-load model by Wells, metabolic-load (e.g. accumulated FM) may challenge metabolic homeostasis (e.g. promote insulin resistance) if it is not resolved by the metabolic capacity for instance through pancreatic insulin production (53). Thus, viewing our findings through the lens of the capacity-load model, it is possible that accelerated weight gain has resulted in elevated levels of C-peptide, insulin and HOMA-IR through an elevated metabolic load (e.g. accumulated FM). Ethiopia and most other Sub-Saharan African countries are currently undergoing a rapid nutritional transition with increased energy availability from the diet (26, 54). Thus, our findings that childhood growth rather than fetal and infant growth was associated with markers of glucose metabolism may reflect an increased exposure to an obesogenic environment, where the excess energy available from the diet is being allocated to weight gain in the form of adipose tissue storage (16), which results in higher levels of circulating insulin and C-peptide. However, as insulin is a peptide hormone

associated with growth stimulation and fat accumulation, we cannot rule out that the associations discussed above is a result of reverse causation (55). Furthermore, it is possible that associations of fetal and infant weight gain with markers of glucose metabolism will appear at later ages.

When adjusting for current weight at 60 mo in model 4 to assess any potential mediation through current weight, the associations of weight gain with blood glucose, C-peptide, insulin, HOMA-IR and LDL cholesterol persisted, but the positive associations with blood pressure disappeared. Interestingly, a new inverse association between birth weight and systolic blood pressure appeared. Correspondingly, studies from both rural and urban India have found that an inverse association between birth weight and systolic blood pressure appeared only when adjusting for current weight (11, 12). This finding may reflect the positive association of birth weight with nephron number (56), an important component of metabolic capacity (57), and the strong association of weight at 60 mo with accumulated FM. Thus, holding constant for weight at 60 mo, those with larger birth weight and more nephrons have lower blood pressure. The positive associations of weight at birth and weight gain in all growth periods with FM and FFM at 60 mo lost significance after adjusting for current weight at 60 mo. However, this was expected due to the close relationship of weight with FM and FFM, and similar results have been seen in comparable studies from high income countries (20, 58).

It should be acknowledged, that none of the children had clinically pertinent levels of any of the cardiometabolic markers, but as cardiometabolic risk markers have been shown to track from childhood to adulthood (59, 60) and adiposity in childhood increase the risk of obesity and cardiometabolic disease in adulthood (61-63), further follow-up studies are needed to

examine how the observed changes in adiposity and cardiometabolic risk markers are developing in later childhood and if accelerated growth after 48 mo really is a risk factor for cardiometabolic risk later in life.

Strengths and limitations

The present study has several important strengths. First, the modelling of growth is based on more than 3300 weight measurements over 12 visits with detailed assessments the first 6 mo of life. Second, the large number of observations and follow-up visits as well as the repeated measurement data structure enabled us to use LSME modelling to estimate the non-linear relationship of weight gain in children. LSME modelling is particularly useful for repeated measures data as it allows for unbalanced and missing data, under an assumption that data are missing at random, as well as accounts for dependencies in the intra-individual weight measurements. Thus, it enabled a more optimal use of the available observations, by allowing participants to be included in the study despite contributing with few weight measurements. This reduced potential bias by selective dropout and increased the statistical power of the study significantly. Third, FM and FFM were measured using an air displacement plethysmograph, which is an accurate, precise and feasible method for assessment of BC in children (37, 38). Lastly, over the 5-year follow-up period with 12 visits, it was possible to include 65% of the 571 children included in the follow-up study after birth in the 5-year follow-up visit. However, we cannot rule out that the observed differences between included and excluded participants could have caused a slight selection bias in the estimated associations (**Supplemental Table 2**). However, our study also has limitations. First, while the LSME modelling can deal with children that have not been measured at all possible visits by allowing those subjects to assume a growth trajectory closer to the

population average, missing visits inevitably makes the estimation of the individual growth trajectories more uncertain. However, in a sensitivity analysis we adjusted our analyses for this uncertainty, and it did not make any difference in the estimated associations (Supplemental Figure 8). Second, for feasibility reasons we standardized the fasting for the 5-year-old children to 3 hours. Not having an overnight fast may have resulted in some non-differential misclassification of the effect sizes, and thus may have caused the effect estimates to move towards zero. Finally, we only included healthy mother-child pairs living in the town of Jimma, which prevents us from generalizing our results to sick or undernourished children, and children living in more rural parts of Ethiopia or rural parts in other sub-Saharan African countries. Whether the findings from this study are reproducible in these populations should be further studied.

Conclusion

Novel statistical methods were used to model weight gain velocities from 0-60 mo, to study the relative importance of weight at birth and weight gain from 0-60 mo with cardiometabolic markers and BC at 60 mo. Our findings suggest that children with a higher weight at 60 mo who gained weight at an accelerated rate, particularly after 48 mo of age, have higher levels of key cardiometabolic markers related to insulin, C-peptide, systolic blood pressure, height, waist circumference, FM and FFM. Collectively, our analyses therefore demonstrate that not all growth periods are equally important for the development of BC and cardiometabolic profile in childhood.

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Author contributions

TG, PK, JCKW, KFM, HF and GSA designed the research; RW, TG, BA, MA, PK, AA, ZG and GSA conducted the research; RW and DV performed the statistical analysis; RW and GSA wrote the paper; All authors revised and approved the final manuscript.

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Tables

Table 1 Background characteristics at birth and at 60 mo of urban Ethiopian children and their mothers for the full sample of children included in the growth modelling and attending the 60 mo follow-up visit [1]

	Full sample (*n* = 375)	**Girls** (*n* = 188)	**Boys** (*n* = 187)	***P* value** [2]	**Missing, *n***
Maternal characteristics					
Age at birth (years)	24.6 ± 4.7	24.9 ± 4.8	24.3 ± 4.7	0.241	0
Postpartum height (cm)	157.1 ± 6.1	157.5 ± 6.2	156.7 ± 5.9	0.212	8
Postpartum body mass index (kg/m^2)	22.23 ± 3.51	22.22 ± 3.43	22.25 ± 3.61	0.947	28
Birth order of current child				0.223	0
First	50.1	46.8	53.5		
Second	26.4	26.1	26.7		
Third or above	23.5	27.1	19.8		
Breast feeding status at 4 to 6 mo post-partum				0.991	43
Exclusive	12.3	12.0	12.7		
Almost exclusive (water given)	21.4	21.0	21.8		
Predominant	60.2	61.1	59.4		
Partial or none	6.0	6.0	6.1		
Maternal education				0.111	0
No school	6.9	5.3	8.6		
Some primary school	44.3	45.2	43.3		
Completed primary school	16.0	20.2	11.8		
Completed secondary school	18.9	16.0	21.9		
Higher education	13.9	13.3	14.4		
Socioeconomic status (International Wealth Index)	45.7 ± 17.3	46.4 ± 17.3	44.9 ± 17.2	0.380	0
Child characteristics at birth					
Gestational age (weeks)	39.0 ± 1.0	39.1 ± 1.0	39.0 ± 1.0	0.384	0
Weight (kg)	3.04 ± 0.41	3.01 ± 0.41	3.07 ± 0.41	0.117	0
Length (cm)	49.1 ± 2.0	48.9 ± 2.0	49.3 ± 2.0	0.055	0
Fat mass (kg)	0.22 ± 0.16	0.23 ± 0.16	0.21 ± 0.17	0.106	2
Fat-free mass (kg)	2.83 ± 0.32	2.77 ± 0.32	2.88 ± 0.32	0.002	2
Low birth weight (%) [3]	9.6	10.6	8.6	0.611	0
Child characteristics at 60 mo					
Age at 60 mo visit (mo)	59.95 ± 1.47	59.89 ± 1.60	60.02 ± 1.33	0.422	0
Weight (kg)	16.38 ± 2.16	16.30 ± 2.22	16.45 ± 2.10	0.509	0
Height (cm)	104.3 ± 4.5	104.2 ± 4.2	104.4 ± 4.7	0.700	0
Weight for age *z*-score [4]	-0.86 ± 0.90	-0.83 ± 0.88	-0.88 ± 0.92	0.613	0
Height for age *z*-score	-1.14 ± 0.92	-1.08 ± 0.86	-1.20 ± 0.98	0.193	0
BMI for age *z*-score	-0.22 ± 0.90	-0.27 ± 0.95	-0.16 ± 0.85	0.270	0
Underweight [5]	9.1	8.5	9.6	0.844	0
Stunted [6]	14.9	12.8	17.1	0.300	0
Wasted by BMI (Thinness) [7]	2.7	4.3	1.1	0.105	0
Overweight [8]	5.1	5.3	4.8	1.000	0
Obese [9]	1.6	1.6	1.6	1.000	0

[1] Values are mean **±** SDs for continues normally distributed variables and percentages for categorical variables. [2] Differences between boys and girls were calculated by One-way ANOVA F-test for continuous variables, Pearson's Chi-Square test of independence for categorical variables with expected counts above 5 in all cells and Fisher's exact test of independence for categorical variables with expected counts in any cell below 5. [3] Low birth weight is defined as birth weight <2500 g. [4] *Z*-scores are derived using the 2006 (children aged <61 mo) and 2007 (children aged ≥61 mo) World Health Organization (WHO) child growth standards (47). [5] Weight for age more than 2 SDs below the age- and sex-specific median of the WHO child growth standards. [6] Height for age more than 2 SDs below the age- and sex-specific median of the WHO child growth standards. [7] BMI for age more than 2 SDs below the age- and sex-specific median of the WHO child growth standards. [8] BMI-for-age from 1 to 2 SDs above the sex-specific median of the WHO child growth standards. [9] BMI-for-age more than 2 SDs above the sex-specific median of the WHO child growth standards.

Table 2 Cardiometabolic markers, anthropometry and body composition at 60 mo of age of urban Ethiopian children and their mothers for the full sample of children included in the growth modelling and attending the 60 mo follow-up visit [1]

	Full sample (*n* = 375)	**Girls** (*n* = 188)	**Boys** (*n* = 187)	***P* value** [2]	**Missing, *n***
Glucose metabolism					
Glucose, whole blood (mmol/L)	5.90 ± 0.84	5.85 ± 0.76	5.96 ± 0.91	0.201	27
HbA1c, whole blood (mmol/mol)	38 ± 4	38 ± 4	38 ± 4	0.789	92
Insulin, serum (µU/mL) [3]	6.03 (3.25-11.20)	7.28 (4.07-12.76)	5.42 (3.02-9.34)	0.004	35
C-peptide, serum (ng/mL) [3]	1.08 (0.68-1.60)	1.16 (0.75-1.90)	0.94 (0.56-1.45)	0.001	40
HOMA-IR [3,4]	1.32 (0.67-2.46)	1.58 (0.85-2.65)	1.12 (0.57-2.09)	0.007	35
Lipids					
Total cholesterol, serum (mmol/L)	3.43 ± 0.61	3.47 ± 0.64	3.38 ± 0.58	0.165	31
LDL cholesterol, serum (mmol/L)	1.66 ± 0.56	1.71 ± 0.56	1.61 ± 0.55	0.097	32
HDL cholesterol, serum (mmol/L)	0.80 ± 0.26	0.79 ± 0.28	0.80 ± 0.24	0.711	36
Triglycerides, serum (mmol/L) [3]	0.96 (0.74-1.29)	0.95 (0.76-1.31)	0.97 (0.72-1.28)	0.605	36
Blood pressure					
Systolic (mmHg)	87.9 ± 7.3	88.1 ± 7.1	87.7 ± 7.5	0.577	2
Diastolic (mmHg)	54.4 ± 8.5	54.7 ± 8.5	54.1 ± 8.4	0.472	2
Anthropometry and body composition					
Body mass index (kg/m^2)	15.02 ± 1.29	14.98 ± 1.44	15.06 ± 1.13	0.570	0
Waist circumference (cm)	51.6 ± 3.2	51.4 ± 3.4	51.8 ± 2.9	0.314	1
Fat mass (kg)	4.19 ± 1.32	4.19 ± 1.46	4.19 ± 1.16	0.991	18
Fat-free mass (kg)	12.20 ± 1.45	12.10 ± 1.36	12.29 ± 1.54	0.216	18
Fat mass index (kg/m^2) [5]	3.84 ± 1.12	3.84 ± 1.23	3.83 ± 0.98	0.916	18
Fat-free mass index (kg/m^2) [5]	11.20 ± 0.88	11.15 ± 0.91	11.26 ± 0.84	0.261	18

[1] Values are mean **±** SDs for continues variables that are normally distributed and median (interquartile range) for continuous variables that are not following a normal distribution. [2] Differences between boys and girls were calculated by One-way ANOVA F-test for continues normally distributed variables. Variables found not to follow a normal distribution were log transformed prior to the tests of group differences. [3] Nonnormally distributed. [4] Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as insulin (µU/mL) × glucose (mmol/l) / 22.5. [5] Fat mass index and Fat-free mass index were calculated by dividing FM and FFM with the squared height in meter, respectively.

Table 3 Average estimated weight at birth, weight gain velocities from birth to 60 mo estimated with linear-spline mixed effects modelling and observed weight at the 60 mo visit in Ethiopian urban children [1]

	Full sample (*n* = 375)	**Girls** (*n* = 188)	**Boys** (*n* = 187)	**WHO growth standards** [2]	
				Girls	**Boys**
Weight (g) Birth visit	3,048 ± 293	3,008 ± 282	3,089 ± 300	3,232	3,346
Weight gain velocity (g/mo) 0-3 mo	1,012 ± 165	984 ± 153	1,041 ± 173	871	1,010
Weight gain velocity (g/mo) 3-6 mo	516 ± 148	506 ± 153	527 ± 143	484	519
Weight gain velocity (g/mo) 6-24 mo	206 ± 49	206 ± 50	206 ± 48	232	234
Weight gain velocity (g/mo) 24-48 mo	140 ± 38	141 ± 38	140 ± 37	191	175
Weight gain velocity (g/mo) 48-60 mo	139 ± 50	143 ± 58	134 ± 40	179	166
Observed weight (g) 60 mo visit	16,378 ± 2,161	16,304 ± 2,223	16,452 ± 2,100	18,219	18,337

[1] Values are mean **±** SDs. [2] Weight gain velocities (g/mo) were calculated by the difference in the estimated median weight in g at the end of the age interval and at the beginning of the age interval divided by the length in mo of the age interval using the Worlds Health Organization 2006 growth standards (47).

Legends for figures

Figure 1. Flow diagram of the study participants and number of children at each follow-up visit from birth to 60 mo of age.

Figure 2. The median weight gain velocity curve for the study population estimated from linear spline mixed effects modelling. The dashed lines show the 95% prediction interval. The vertical grey lines show the selected knot points, and the slopes between the knot points express the estimated median weight gain velocity in each of the age intervals. The shaded grey areas show the reference in standard deviation scores from the median weight-for-age according to the World Health Organization international growth standards.

Figure 3. Associations of estimated birth weight, weight gain velocities and observed weight at 60 mo with height, fat-free mass and markers of adiposity (A) and cardiometabolic risk (B). The coefficients (and 95% CI) displayed on the forest plot were derived from separate multiple linear regression models and represent the change in the 60-mo outcomes per study population standard deviation score increase in estimated birth weight, weight gain velocities and observed weight at the 60-mo visit, respectively. The linear spline mixed effects model used to estimate birth weight and weight gain velocities had 4 internal knot points at 3, 6, 24 and 48 mo, yielding the 5 growth periods 0-3, 3-6, 6-24, 24-48 and 48-60 mo. Weight at 60 mo was standardized prior to the analyses. Nonnormally distributed variables (i.e. insulin, C-peptide, HOMA-IR and triglycerides) were log transformed prior to the regression analysis. The resulting effect

estimates were back-transformed and presented as percentwise change. Model 1 was adjusted for sex, birth order and gestational age. Model 2 was additionally adjusted for the child's exact age at the 60-mo visit, maternal age at delivery, maternal postpartum height, maternal educational status and family socioeconomic status (International Wealth Index). Model 3 was additionally adjusted for child birth weight. Model 4 was additionally adjusted for child weight at 60 mo. In the analyses of the outcomes systolic and diastolic blood pressure model 4 was adjusted for height and weight at 60 mo in addition to the adjustments in model 3. The analyses of estimated birth weight as primary predictor did not include a model 3 and model 4, in these analyses, was in addition to the adjustments in model 2 adjusted for child weight at 60 mo. The analyses of weight at 60 mo as primary predictor did not include a model 4 for all outcomes except for systolic and diastolic blood pressure, where model 4 was adjusted for height at 60 mo in addition to the adjustments in model 3. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

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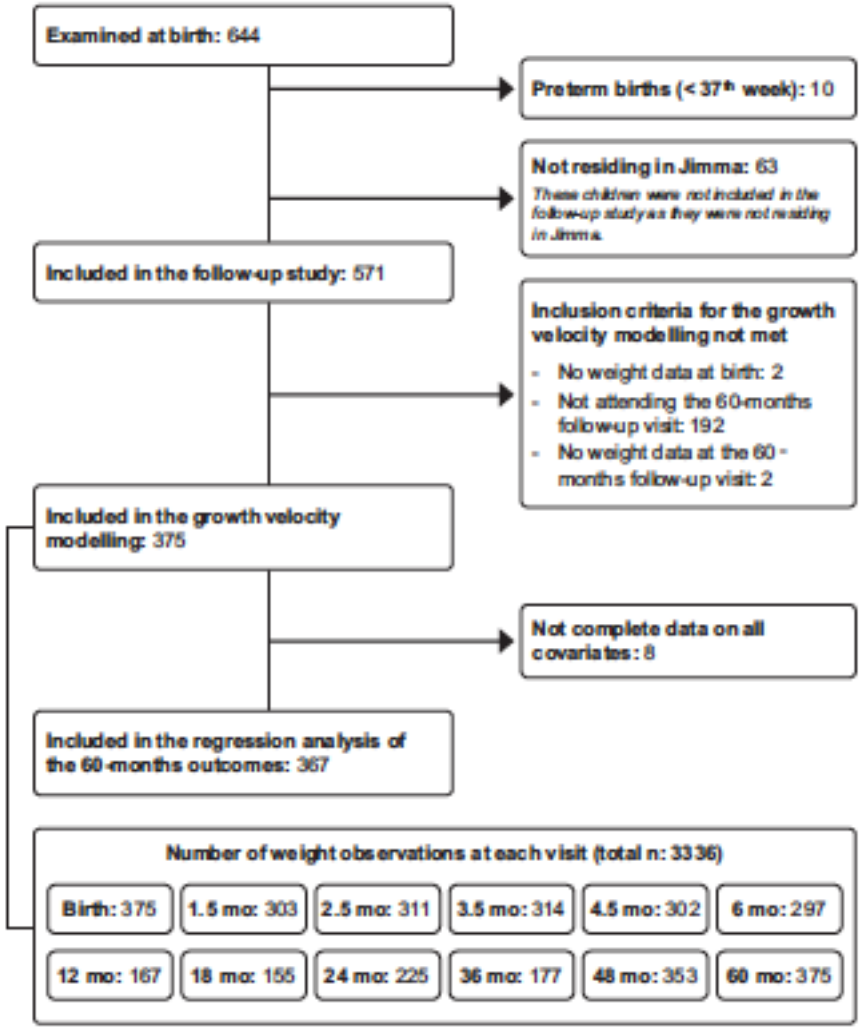
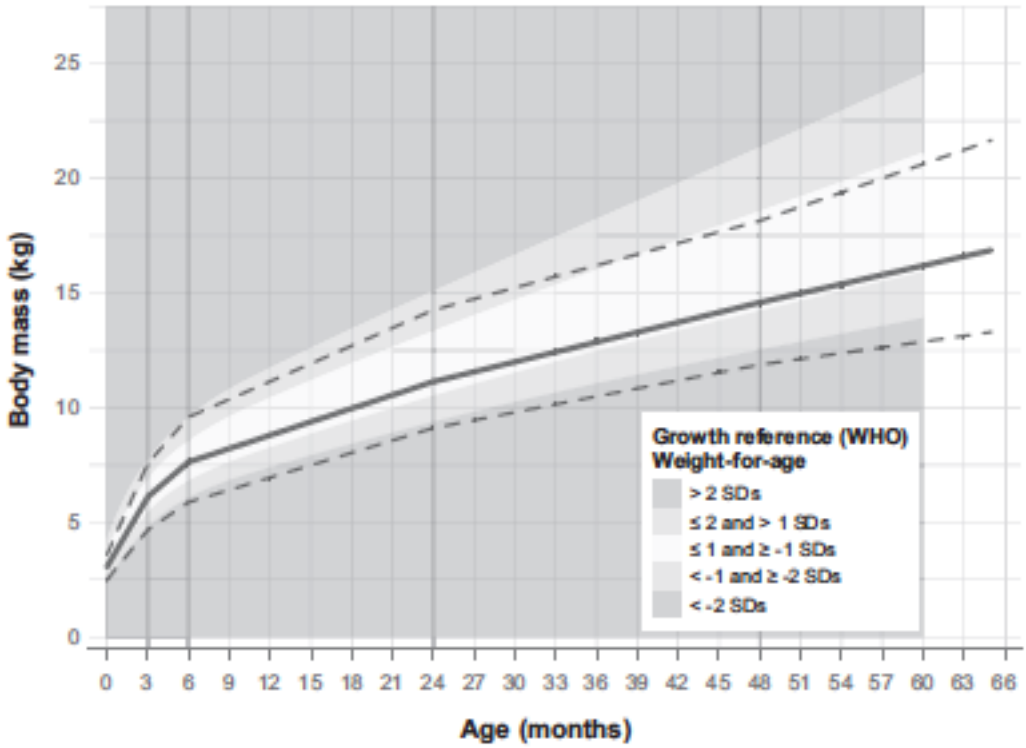
Fig 1


Examined at birth: 644
Preterm births (< 37th week): 10
Not residing in Jimma: 63
These children were not included in the follow-up study as they were not residing in Jimma.
Included in the follow-up study: 571
Inclusion criteria for the growth velocity modelling not met
- No weight data at birth: 2
- Not attending the 60-months follow-up visit: 192
- No weight data at the 60 - months follow-up visit: 2
Included in the growth velocity modelling: 375
Not complete data on all covariates: 8
Included in the regression analysis of the 60-months outcomes: 367
Number of weight observations at each visit (total n: 3336)
Birth: 375
1.5 mo: 303
2.5 mo: 311
3.5 mo: 314
4.5 mo: 302
6 mo: 297
12 mo: 167
18 mo: 155
24 mo: 225
36 mo: 177
48 mo: 353
60 mo: 375

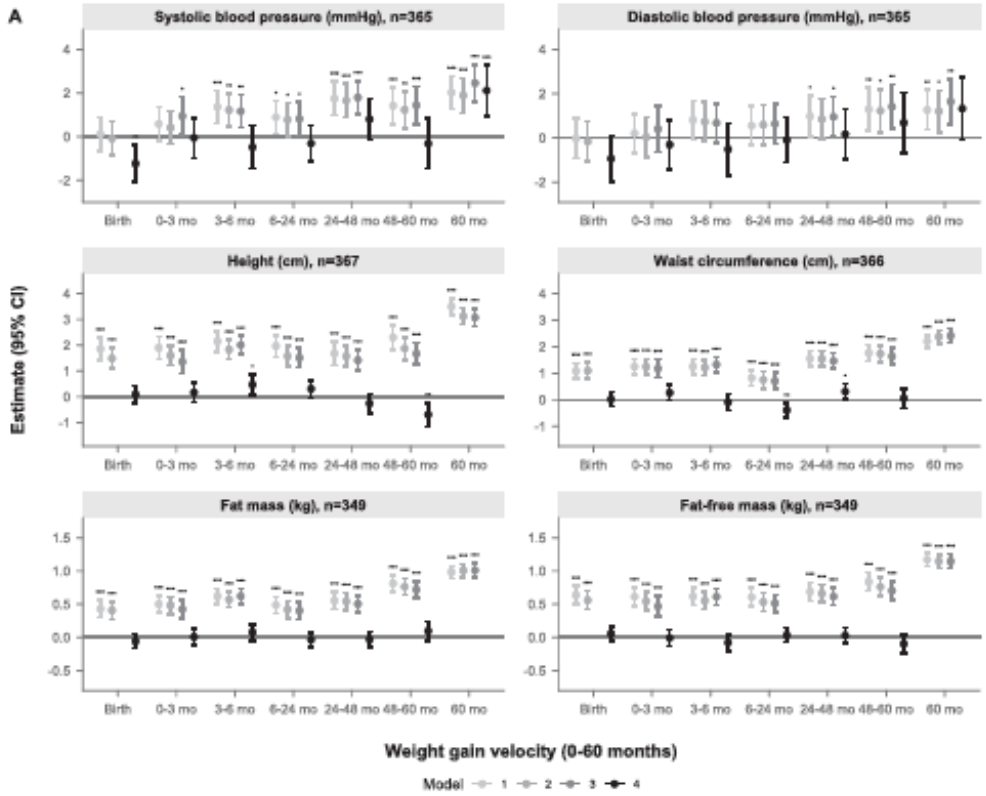
444

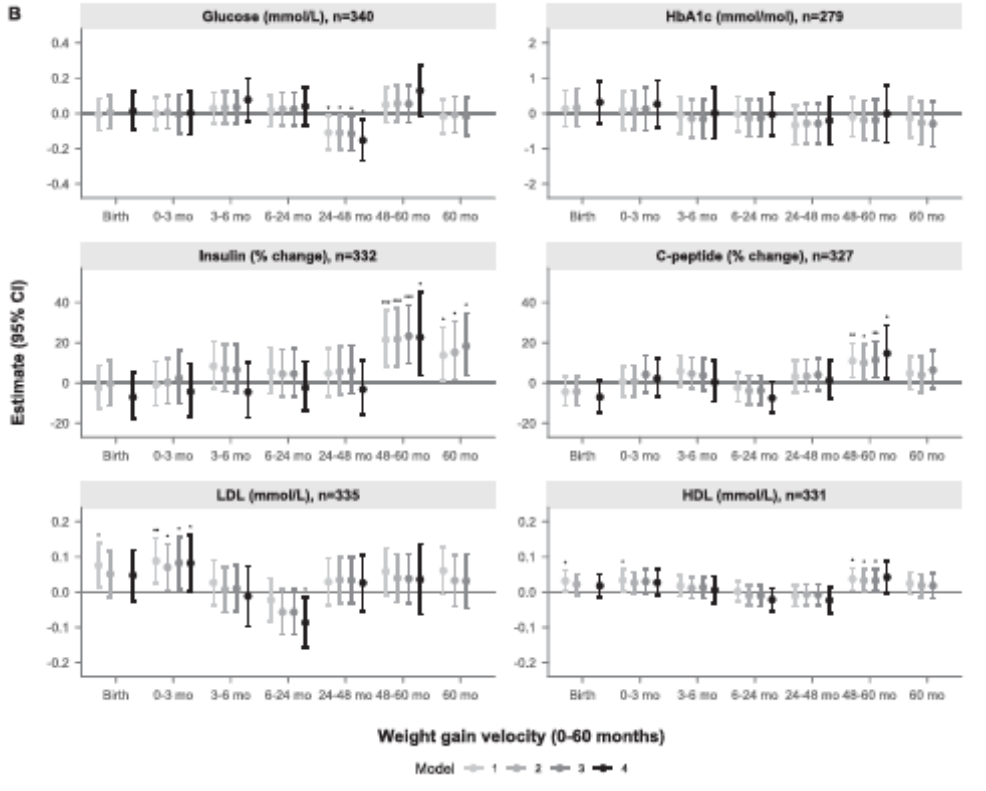
Fig 2


Body mass (kg)
25
20
15
10
5
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66
Age (months)
Growth reference (WHO)
Weight-for-age
> 2 SDs
≤ 2 and > 1 SDs
≤ 1 and ≥ -1 SDs
< -1 and ≥ -2 SDs
< -2 SDs

445

Fig 3


A
Systolic blood pressure (mmHg), n=365
Diastolic blood pressure (mmHg), n=365
Height (cm), n=367
Waist circumference (cm), n=366
Fat mass (kg), n=349
Fat-free mass (kg), n=349
Estimate (95% CI)
Birth
0-3 mo
3-6 mo
6-24 mo
24-48 mo
48-60 mo
60 mo
Weight gain velocity (0-60 months)
Model 1 2 3 4


B
Glucose (mmol/L), n=340
HbA1c (mmol/mol), n=279
Insulin (% change), n=332
C-peptide (% change), n=327
LDL (mmol/L), n=335
HDL (mmol/L), n=331
Estimate (95% CI)
Birth
0-3 mo
3-6 mo
6-24 mo
24-48 mo
48-60 mo
60 mo
Weight gain velocity (0-60 months)
Model 1 2 3 4