TITLE: Body Composition Growth Patterns in Early Infancy: A Latent Class Trajectory Analysis of the Ethiopian iABC Birth Cohort

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?
Fetal and infant growth when assessed through simple anthropometry is linked to later risk of obesity, type 2 diabetes and cardiovascular disease.

There is a substantial variation in birth and infant body composition not reflected in crude weight or length measures.

Low birth weight is commonly followed by catch-up growth

WHAT DOES THIS STUDY ADD?
Identifies novel subgroups with distinct fat and fat-free mass growth patterns in early infancy.

Shows that underlying the already known anthropometric descriptions of infant growth, there are heterogeneous patterns of fat and fat-free growth that have not previously been identified and are not detectable with anthropometric measures.

Shows that catch-up fat growth is the primary driver underlying the catch-up growth previously described with simple anthropometric measurements.

Describe predictors of these distinct body composition growth patterns that are relevant for future interventions and points at future research needs.

Abstract

Objective: To identify subgroups with distinct fat and fat-free growth patterns in the first six months of life and describe predictors of these different patterns.

Methods: A total of 510 apparently healthy Ethiopian infants were followed from birth to six months of age. Each infant had at least three and up to six repeated measurements of fat- and fat-free mass using air-displacement plethysmography. Latent class trajectory analyses were used to categorize infants in groups with distinct body composition patterns.

Results: Four distinct fat mass and two fat-free mass growth patterns were identified. Five % of the infants represented a *delayed fat* growth pattern and 3% a *catch-up fat* growth pattern involving low birth weight but a significant fat growth velocity from 2.5-6 months. A large class had a high fat level at birth and an *accelerated fat* growth pattern in early infancy. Fat-free growth was represented by two distinct classes with less variability. Catch-up growth was primarily seen in fat mass.

Conclusions: We identified distinct patterns of delayed, catch-up, and accelerated fat growth in early infancy. This variability is not detected in regular anthropometric assessment, and could be a mechanism linking early growth with later obesity and cardio-metabolic risk.

Introduction

Fetal and infant growth has important implications for health and disease through the life course. Birth weight is a commonly used marker of fetal growth and studies have shown that low birth weight predicts childhood morbidity and mortality in low-income countries (1, 2) and is associated with an increased risk of obesity, type 2 diabetes (T2D) and cardiovascular diseases (CVD) later in life (3, 4, 5). Infants born small for gestational age typically experience catch-up growth in infancy. This is critical for survival and healthy development in the short term (6), but has also been associated with increased adult risk of T2D and CVD (7, 8, 9, 10). Contrasting with these findings, analyses conducted in historical cohorts have shown that low body weight at one year also predicted the same diseases (11, 12).

These complex associations between early life growth patterns and short and long-term health outcomes are likely to derive in part from variability in the underlying patterns of fat and lean tissue accretion (13, 14, 15, 16). Dulloo et al. presented a case for this in the "thrifty catch-up fat phenotype" model, suggesting that catch-up growth in infancy and childhood favours fat growth and argued that the processes promoting preferential catch-up fat increases susceptibility to development of insulin resistance (17). However, due to a lack of appropriate methods, it only recently became feasible to study in humans the individual variability in the early life accretion of fat and lean tissues, and the "thrifty catch-up fat phenotype" model has not been tested in a Sub-Saharan African population. From a prospective cohort study in apparently healthy Ethiopian infants, we recently provided population-level body composition (BC) reference data (18) and demonstrated a remarkable variability in fat and fat-free mass in newborns with similar weight (19), but it is not known how neonatal BC heterogeneity tracks into infancy, and how catch-up growth is reflected in fat- and fat-free tissue accretion. Identification of subgroups with distinct infant fat and lean tissue accretion patterns is

therefore essential to further understand how early life growth patterns relate to health consequences through the life course.

One approach to identify subgroups with common patterns is to apply data-driven statistical methods such as latent class trajectory analyses (20, 21). This method allows for identification of distinct subgroups of infants who are homogeneous with respect to the development over time, but heterogeneous as compared with other groups. In the present study we apply latent class trajectory analyses to identify subgroups with distinct fat and fat-free mass growth patterns in early infancy and describe predictors of these subgroups.

Methods

Study setting and participants

Mothers and their newborn infants were recruited among women giving birth at Jimma University Specialized Hospital (JUSH), Ethiopia from December 2008 to October 2012. Women were considered eligible if they were living in Jimma town, and had given birth at the JUSH maternity ward to a child with a birth weight (BW) of ≥1500 gram. If consent was given, women and newborns were examined shortly after birth, while still admitted to the hospital and no later than 48 hours after birth.

Examinations of the mothers and infants

All measurements and interviews were performed by trained research nurses. Information on maternal background characteristics was obtained through questionnaires presented to the mothers at the birth examination, which also included estimation of gestational age by physical examination of the newborn according to the New Ballard score standard (22). The

baseline examination of women and newborns was conducted shortly after birth while still admitted to the hospital. In total, 93.4% of the birth examinations were performed within 24 h after birth and the remaining no later than 48 h after birth, to assure the highest precision in the New Ballard score. Infants with medical conditions or edema detected during the baseline examination were not included in the study, but admitted to the neonatology unit at JUSH. In case of observed medical conditions or significant growth failure during any of the follow-up examinations, a paediatrician was called for further clinical examination and if relevant the infant was referred for further management. Details on data collection procedures have previously been described in more detail (18)

Anthropometry and body composition

Infant length, weight and BC was measured at all examinations. Infant length was measured to the nearest 0.1 cm using a SECA 416 Infantometer (SECA, Hamburg, Germany). Weight, fat- and fat-free mass was done with the PEA POD® (COSMED, Rome, Italy), an infant air-displacement plethysmograph (ADP). Infant ADP has been validated in other studies (23, 24) and in a subsample of the current cohort (18), and shown good accuracy and high precision. During the 2-min ADP measurement, infants were lying on a plastic bed in a closed compartment without clothes wearing only a wig pap. The principles and design of the infant ADP instrument has been reported in detail elsewhere (25), and we have previously described how ADP measurements were onducted in the present cohort (18, 19)

Maternal weight, fat- and fat-free mass was measured to the nearest 0.1 kg using a Tanita 418 (Tanita Corp., US) bio impedance analyser, and height to the nearest 0.1 cm using a SECA 214 stadiometer (SECA, Hamburg, Germany). All anthropometry was measured in duplicate, and the average of double measurements used for analysis.

Follow-up procedures

After birth examination mothers and infants were appointed for further examinations at 1.5, 2.5, 3.5, 4.5 and 6 months of age. Participants failing to show up were contacted by telephone or visited at their home, and asked if they were willing to appear for examination. For this reason, there is some variation in the actual age at assessment across the study period, and few infants were measured up to the age of 8 months of age.

Ethics

Written informed consent was obtained from all participating women. Thorough information about the study and examinations was given orally and written in local language. Women were free to retract from the study at any time. Any serious medical conditions observed by the research nurses was addressed in accordance with local recommendations. Ethical permission was granted from Jimma University Ethical Review Committee. The study is registered in ISRCTN (https://www.isrctn.com/): *ISRCTN46718296*

Data handling and statistical analysis

Baseline infant and maternal characteristics based on continuous variables were presented as means (SD) and categorical variables as percentages (n).

The latent class trajectory analysis grouped the study population into classes by evaluating similarities in their BC measurements over time so that participants in the same class had similar trajectories of BC change. Within each class, the repeated BC measurements were modelled by a linear mixed-effects model with fat mass or fat-free mass as the dependent variable and infant age as independent variable. The longitudinal trajectories of fat mass and

fat-free mass were described by infant age using cubic polynomials (natural cubic splines) to allow the trajectories to develop non-linearly across age (26). We identified four age-points (knots) with a high data density and fitted cubic polynomials between each adjacent age-point.

To choose the optimal number of latent classes to describe the heterogeneity in BC development, we compared fit of models with different number of latent classes using the Bayesian Information Criterion (BIC). For each participant, the latent class trajectory model calculates a posterior probability of membership in each class, and each participant is assigned to the class for which his/her posterior probability is the highest. We required a minimum of 3% of the infants in each latent class. Only infants with at least three BC measurements were included in the latent class models. Further model specifications are given as supporting information.

To examine whether maternal and infant characteristics differed by latent class, we applied general linear models when characteristics variables were continuous and logistic regression when variables were categorical. All models were adjusted for infant sex and maternal age. To test whether sex modified the association between maternal and infant characteristics and latent class, models with and without sex included as an interaction term was compared using a likelihood ratio test. If the overall test indicated significant differences in means or proportions by latent class ($p<0.05$), pairwise differences were studied with post hoc comparison of contrasts using the *pwcompare* function in Stata 14.0 (StataCorp, Texas, USA). Finally, to describe fat mass growth for each fat-free mass latent class and fat-free mass growth for each fat mass latent class, we applied two additional standard linear mixed-effects models. I.e. to describe fat mass growth for each fat-free mass latent class we defined a linear

mixed-effects model with fat mass as the dependent variable, and infant age and the fat-free mass latent classes as the independent variables. Infant age was modelled as described for the latent class trajectory models.

Analyses were performed in Stata 14.0 and 15.1 (StataCorp, Texas, USA) and R version 3.2.3 (The R foundation for Statistical Computing).

Results

A total of 644 women accepted the invitation to participate in the study and were examined together with their infants. Ten infants were born before the 37th week of gestation and 101 were only measured once at birth and lost to follow-up shortly thereafter. A further 22 had less than three measurements in the study period. These were all excluded. At birth and during the six months follow-up, the remaining 510 infants had a total of 2 682 valid BC, anthropometry and questionnaire assessments performed. Three of the 510 BC measurements at birth were invalid and the BC measurements were distributed across the six visits as follows: 507 BC measurements at the birth visit, 445 at 1.5 months, 449 at 2.5, 453 at 3.5, 433 at 4.5 and 395 at the 6 months visit. **Figure 1** shows a flow chart of the study participants. The main reasons for loss-to follow up in the iABC cohort at each visit has previously been described in detail and relates to moving away from the study site shortly after giving birth in the majority of cases (69%) (18). There were no differences in BC at birth between infants followed and infants lost to follow-up (18). Baseline maternal and infant characteristics are presented in **Table 1.**

Body composition patterns

Based on the latent class models we identified four distinct fat growth patterns **(Figure 2)** and two distinct fat-free growth patterns **(Figure 3)** from birth to 6 months of age.

The "*Intermediate fat"* latent class represented 59.4% (n=303) of the total sample and was characterised by a pattern roughly following the 50th percentile growth curve previously reported from this cohort (18). The second largest *"Accelerated fat"* class comprised 32.4% (n=165) of the infants and showed a high birth fat level and a high initial fat growth velocity in the first months of life with a flattening at around 4 months of age at a high fat level. A third "*Delayed fat"* class captured 5.3% (n=27) of the infants and was characterised by a low birth fat level and low initial velocity, resulting in a low fat level throughout the period. Finally, a fourth class comprising 3.0% (n=15) of the infants were at a low birth fat level but showed a significant fat catch-up pattern from 2.5-6 months, ending up at the highest fat mass level at 6 months of age. We named this class the *"Catch-up fat"* pattern**.** The overall population fat growth pattern followed a pattern largely parallel to the intermediate fat growth pattern but at a slightly higher level throughout (Supporting Information Figure S1).

Two distinct fat-free mass growth patterns were identified. A class representing 44.7% (n=228) of the infants had a lower initial fat-free mass level and an almost linear fat-free mass pattern throughout early infancy whereas the other class of 55.3% (n=282) demonstrated a higher initial fat-free mass level, a more quadratic shaped pattern, and a higher 6 months fat-free mass level. We named these the *"Linear fat-free"* and "*Quadratic fat-free*" classes, respectively. The overall population fat-free growth pattern followed a pattern almost exactly in between the linear and the quadratic growth patterns (Supporting Information Figure S2).

To describe relations between fat mass growth and fat-free mass growth we also modelled for each fat growth class the corresponding fat-free growth pattern, and for each fat-free growth class the corresponding fat growth pattern (Supporting Information Figure S3-S4). Fat-free growth patterns for the 4 fat classes were largely parallel and linear, except for fat-free growth in the catch-up fat class which showed a modest catch-up in fat-free growth although not to the extent seen for fat. Fat growth patterns for the two fat-free classes largely followed the population average fat growth trajectory, but infants in the quadratic fat-free class had a higher fat growth velocity.

For each class we calculated the average of the individuals' posterior probability of class membership. The posterior probability can be used to assess the discrimination of the latent classes. The average probabilities were high for all classes ranging from 79%–89% (27). The average probabilities for each class are provided in **table 2**.

Maternal and infant predictors

To identify predictors of the BC patterns, we compared the distribution of baseline maternal and infant characteristics across the latent classes in **Table 3**. Birth weight, length, fat and fat-free mass were significantly higher in the accelerated fat class compared to all other fat classes, and the delayed fat class had the lowest levels of birth weight, length and fat-free mass and significantly lower than the intermediate fat class. Birth weight, length and fat-free mass were also significantly lower in the linear fat-free class compared to the quadratic class. Around 20% of the infants in the catch-up and delayed fat class were born with a low birth weight (<2 500g), compared to only 10.9% and 2.4% in the intermediate and accelerated fat classes, respectively. Fewer infants were also low birth weight in the quadratic fat-free class, compared to the linear fat-free class. Females were more likely to belong to the linear fat-free

class, but there were no significant sex differences across the fat classes, and sex did not modify the association between fat- or fat-free class memberships and any of the maternal and infant characteristics. First born infants were more likely to show a catch-up fat pattern compared to the other fat classes. Exclusive breastfeeding was associated with a quadratic fat-free growth pattern whereas no breastfeeding was associated with a delayed fat growth pattern. Finally, infants in the delayed fat class were less likely to belong to the quadratic fat-free class compared to the intermediate and accelerated fat classes.

Discussion

We have identified four distinct fat growth patterns and two distinct fat-free patterns in the first 6 months of life, based on data-driven latent class trajectory analyses, and found that at number of maternal and infant factors were associated with these BC patterns. The findings in the present study extend the current knowledge by identifying novel subgroups with significant heterogeneity in BC growth in early infancy. Other studies have presented population level data on BC growth in healthy infants (28, 29, 30, 31, 32). The current study differs in that we apply a data driven approach to identify non typical but potentially clinically important longitudinal BC accretion patterns in infancy that are not detectable in studies reporting BC growth as age-specific averages.

Body composition trajectories

The identification of a rare but pronounced catch-up fat accretion pattern and less indication of catch-up in fat-free mass is interesting. A rapid postnatal catch-up in weight for length has been suggested to increase the risk of type-2 diabetes, CVD and obesity later in life (7, 8, 9). The BC patterns presented in the present study suggest that the catch-up may occur particularly in fat mass. This is supported by rodent studies showing that in a calorie restricted

environment, energy conservation favours adipose tissue and leads to accretion of fat mass rather than muscle mass during catch-up growth which leads to stimulation of hyperinsulinemia and pancreatic β-cell hyper responsiveness to glucose through a pathway of mechanisms involving suppressed thermogenesis and glucose oxidation in skeletal muscle (13, 14, 15, 16, 17). Our findings provide support to a preferential catch-up of fat in a subpopulation with lower birth weights. Follow-up with blood samples of this and similar cohorts may uncover whether the catch-up fat pattern is also linked to glucose metabolism and insulin sensitivity, as suggested by Dulloo et al (17).

The catch-up fat growth pattern contrasts the delayed fat pattern throughout early infancy despite having similar low fat mass levels at birth and high proportions of low birth weight infants. Background factors for these two groups were similar in most aspects with some exceptions. First born infants were more likely to belong to the catch-up fat class. Other studies have shown a lower birth weight but higher growth velocity in first born infants (33). Our findings add to the current knowledge by suggesting that a high infant growth velocity in first borns is fuelled primarily by increased fat rather than fat-free growth. We also found that not being breastfed at 3 months of age was associated with a delayed fat growth pattern. This corresponds with findings from a meta-analysis showing that formula-fed infants had lower fat mass at 3-4 and 6 months of age compared with breastfed infants (34). However, our findings are based on very few infants as the vast majority in our study were fully or predominantly breasted and the delayed fat growth group is small. Furthermore, not all not-breastfed infants in the present study were formula-fed but rather cow-milk fed or a combination of the two. Finally, not being breastfed in the present study may represent undetected underlying clinical conditions that may also affect body composition. Thus, these findings should be interpreted with caution.

We identified a large proportion of infants with an accelerated fat growth pattern in a Sub-Saharan African (SSA) infant population. It is known from studies in non-SSA populations that accelerated or adiposity growth patterns in infancy tracks throughout childhood and adolescence, and is associated with obesity, T2D and CVD in adulthood (35, 36). Our findings indicate that a similar patterns may exist in a Sub-Saharan African population undergoing a significant nutritional transition (37).

The two fat-free mass patterns differed in that they started at different levels of fat-free mass at birth. The linear trajectory largely followed the 50th fat-free growth percentile previously shown for this cohort (18), and the quadratic curved fat-free mass trajectory were at an initial higher fat-free mass level at birth, followed a slightly quadratic shaped growth curve and reached a higher level at 6 months. In these first months of life skeletal muscle is still undergoing development, and it has been suggested that slow muscle growth in this period could augment later insulin resistance (38). Long-term follow up with assessment of glucose metabolism and insulin resistance is needed to address this hypothesis further.

In previous studies, when growth in infancy assessment is based on simple anthropometry, both accelerated, delayed and catch-up growth patterns have been associated with a number of conditions later in life, including obesity, T2D and CVD (39, 40, 41, 42, 43, 44). A possible explanation for these conflicting associations is that they reflect different types of undetected variability in fat- and muscle mass accretion. In example, slow muscle mass growth in early infancy may present as a pattern of delayed growth when measured with simple anthropometry and may also affect later insulin resistance (38). In contrast, a catch-up fat growth pattern may mask as a high general weight gain in infancy and has also been

suggested to affect hyperinsulinemia and pancreatic β-cell hyper responsiveness to glucose (17).

Strengths and weaknesses

A strength in this study is the use of a data driven approach to identify heterogeneous BC growth patterns. Most existing data on BC development in early infancy is based on the assumption of an average pattern of BC development through infancy applicable for the whole study population (18, 28, 29, 30, 31, 32, 45), and consequently overlook potentially important and distinct but less common BC growth patterns. Other data driven approaches are available to identify similarities and relationships in data including various cluster-analyses approaches, but few of these allows taking into account several repeated measurements on the same individuals.

Another strength is the use of a precise and accurate method for assessment of infant BC in a relatively large dataset with repeated measurements and >2 500 datapoints. Air displacement plethysmography has been evaluated against gold standard BC methods in several populations, including a subsample of the iABC cohort used for the present analyses, and was found to be safe, accurate and precise (18, 23, 24, 25).

A weakness of the latent class analysis approach is that the researcher has little control over the size of the subgroups and modelling may result in small sizes of subgroups, and consequently limited power to make statistical inferences or limited relevance to generalize results. The catch-up and delayed fat growth classes were small, but may represent important and clinically relevant growth patterns. However, this should be confirmed in other infant BC cohorts. We estimated gestational age by physical examination of the newborn according to

the New Ballard score standard (22). When validated against prenatal ultrasonography, the New Ballard Score was found to be valid and accurate with a mean difference of 0.15±1.46 wks. Still, ultrasonography is a more robust approach and it is possible that some newborns were misclassified in terms of gestational age. Since this study only included term infants, this is however, not likely to have a large impact on the findings presented. Although we identified a number of maternal and infant factors that were associated with the BC growth patterns, there are other factors that we were unable to address in the present study. These include maternal nutrition in pregnancy, known to affect fetal development and birth characteristics. Thus, it is possible that suboptimal maternal nutrition in pregnancy affects the presented BC growth patterns and that maternal nutrition in pregnancy is different in this Ethiopian population compared to other more affluent populations. On the other hand, this study was performed in a population of urban living, healthy infants without a known history of fetal growth restriction or a particular obesogenic fetal environment. In a different environment where fetal growth may be impeded or overstimulated, these growth patterns could be more common. Still, birth weight and fat mass levels in this Ethiopian cohort are lower than seen in some other infant BC studies (19), and generalization to high-income infant populations should be done with caution.

Conclusion

We used latent class analysis to identify and characterize four distinct fat mass growth patterns and two distinct fat-free mass growth patterns in the first 6 months of life in Ethiopian infants, and uncovered a significant heterogeneity in early infant BC growth patterns, which was also associated with a number of maternal and infant factors. Long term follow-up of this and similar infant BC cohorts may clarify to which extent patterns of catch-

up, accelerated or delayed fat growth affects childhood development of insulin resistance, adiposity and cardio-metabolic risk factors, and adult development of obesity, T2D and CVD.

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References

1. Ashworth A. Effects of intrauterine growth retardation on mortality and morbidity in infants and young children. *EurJClinNutr* 1998;**52 Suppl 1:** S34-S41.

2. Shrimpton R. Preventing low birthweight and reduction of child mortality. *TransRSocTropMedHyg* 2003;**97:** 39-42.

3. Barker DJ, Eriksson JG, Forsen T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. *IntJEpidemiol* 2002;**31:** 1235-1239.

4. Levitt NS, Lambert EV, Woods D, Hales CN, Andrew R, Seckl JR. Impaired glucose tolerance and elevated blood pressure in low birth weight, nonobese, young south african adults: early programming of cortisol axis. *J Clin Endocrinol Metab* 2000;**85:** 4611-4618.

5. Forrester TE, Wilks RJ, Bennett FI, Simeon D, Osmond C, Allen M*, et al.* Fetal growth and cardiovascular risk factors in Jamaican schoolchildren. *BMJ* 1996;**312:** 156-160.

6. Victora CG, Barros FC, Horta BL, Martorell R. Short-term benefits of catch-up growth for small-for-gestational-age infants. *Int J Epidemiol* 2001;**30:** 1325-1330.

7. Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? *The Lancet* 2004;**363:** 1642-1645.

8. Kerkhof GF, Leunissen RWJ, Hokken-Koelega ACS. Early origins of the metabolic syndrome: role of small size at birth, early postnatal weight gain, and adult IGF-I. *J Clin Endocrinol Metab* 2012;**97:** 2637-2643.

9. Fabricius-Bjerre S, Jensen RB, Færch K, Larsen T, Mølgaard C, Michaelsen KF*, et al.* Impact of birth weight and early infant weight gain on insulin resistance and associated cardiovascular risk factors in adolescence. *PloS one* 2011;**6**.

10. Stettler N, Stallings VA, Troxel AB, Zhao J, Schinnar R, Nelson SE*, et al.* Weight gain in the first week of life and overweight in adulthood: a cohort study of European American subjects fed infant formula. *Circulation* 2005;**111:** 1897-1903.

11. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C*, et al.* Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 1991;**303:** 1019-1022.

12. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet* 1989;**2:** 577-580.

13. Jimenez-Chillaron JC, Hernandez-Valencia M, Lightner A, Faucette RR, Reamer C, Przybyla R*, et al.* Reductions in caloric intake and early postnatal growth prevent glucose intolerance and obesity associated with low birthweight. *Diabetologia* 2006;**49:** 1974-1984.

14. Jimenez-Chillaron JC, Patti M-E. To catch up or not to catch up: is this the question? Lessons from animal models. *Curr Opin Endocrinol Diabetes Obes* 2007;**14:** 23-29.

15. Cettour-Rose P, Samec S, Russell AP, Summermatter S, Mainieri D, Carrillo-Theander C*, et al.* Redistribution of Glucose From Skeletal Muscle to Adipose Tissue During Catch-Up Fat A Link Between Catch-Up Growth and Later Metabolic Syndrome. *Diabetes* 2005;**54:** 751-756.

16. Casimir M, de Andrade PB, Gjinovci A, Montani JP, Maechler P, Dulloo AG. A role for pancreatic beta-cell secretory hyperresponsiveness in catch-up growth hyperinsulinemia: Relevance to thrifty catch-up fat phenotype and risks for type 2 diabetes. *Nutr Metab (Lond)* 2011;**8:** 2.

17. Dulloo AG, Jacquet J, Seydoux J, Montani JP. The thrifty 'catch-up fat' phenotype: its impact on insulin sensitivity during growth trajectories to obesity and metabolic syndrome. *Int J Obes (Lond)* 2006;**30 Suppl 4:** S23-35.

18. Andersen GS, Girma T, Wells JC, Kaestel P, Leventi M, Hother AL*, et al.* Body composition from birth to 6 mo of age in Ethiopian infants: reference data obtained by air-displacement plethysmography. *The American journal of clinical nutrition* 2013;**98:** 885-894.

19. Andersen GS, Girma T, Wells JC, Kaestel P, Michaelsen KF, Friis H. Fat and fat-free mass at birth: air displacement plethysmography measurements on 350 Ethiopian newborns. *Pediatric research* 2011;**70:** 501-506.

20. Proust-Lima C, Letenneur L, Jacqmin-Gadda H. A nonlinear latent class model for joint analysis of multivariate longitudinal data and a binary outcome. *Stat Med* 2007;**26:** 2229-2245.

21. Nagin DS, Odgers CL. Group-based trajectory modeling in clinical research. *Annual review of clinical psychology* 2010;**6:** 109-138.

22. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score, expanded to include extremely premature infants. *JPediatr* 1991;**119:** 417-423.

23. Ma G, Yao M, Liu Y, Lin A, Zou H, Urlando A*, et al.* Validation of a new pediatric air-displacement plethysmograph for assessing body composition in infants. *AmJClinNutr* 2004;**79:** 653-660.

24. Ellis KJ, Yao M, Shypailo RJ, Urlando A, Wong WW, Heird WC. Body-composition assessment in infancy: air-displacement plethysmography compared with a reference 4-compartment model. *AmJClinNutr* 2007;**85:** 90-95.

25. Urlando A, Dempster P, Aitkens S. A new air displacement plethysmograph for the measurement of body composition in infants. *PediatrRes* 2003;**53:** 486-492.

26. Collins GS, Ogundimu EO, Cook JA, Manach YL, Altman DG. Quantifying the impact of different approaches for handling continuous predictors on the performance of a prognostic model. *Stat Med* 2016;**35:** 4124-4135.

27. Nagin DS. *Group-based modeling of development.* . Harvard University Press: Cambridge, MA, USA, 2005.

28. Carberry AE, Colditz PB, Lingwood BE. Body composition from birth to 4.5 months in infants born to non-obese women. *Pediatric research* 2010;**68:** 84-88.

29. Fomon SJ, Haschke F, Ziegler EE, Nelson SE. Body composition of reference children from birth to age 10 years. *AmJClinNutr* 1982;**35:** 1169-1175.

30. Fields DA, Gilchrist JM, Catalano PM, Giannì ML, Roggero PM, Mosca F. Longitudinal body composition data in exclusively breast-fed infants: a multicenter study. *Obesity (Silver Spring)* 2011;**19:** 1887-1891.

31. Butte NF, Hopkinson JM, Wong WW, Smith EO, Ellis KJ. Body composition during the first 2 years of life: an updated reference. *PediatrRes* 2000;**47:** 578-585.

32. Eriksson B, Löf M, Forsum E. Body composition in full-term healthy infants measured with air displacement plethysmography at 1 and 12 weeks of age. *Acta Paediatrica (Oslo, Norway: 1992)* 2010;**99:** 563-568.

33. Pizzi C, Cole TJ, Richiardi L, dos-Santos-Silva I, Corvalan C, De Stavola B. Prenatal influences on size, velocity and tempo of infant growth: findings from three contemporary cohorts. *PloS one* 2014;**9:** e90291.

34. Gale C, Logan KM, Santhakumaran S, Parkinson JR, Hyde MJ, Modi N. Effect of breastfeeding compared with formula feeding on infant body composition: a systematic review and meta-analysis. *The American journal of clinical nutrition* 2012;**95:** 656-669.

35. Araujo J, Severo M, Barros H, Mishra GD, Guimaraes JT, Ramos E. Developmental trajectories of adiposity from birth until early adulthood and association with cardiometabolic risk factors. *Int J Obes (Lond)* 2015;**39:** 1443-1449.

36. Slining MM, Herring AH, Popkin BM, Mayer-Davis EJ, Adair LS. Infant BMI trajectories are associated with young adult body composition. *J Dev Orig Health Dis* 2013;**4:** 56-68.

37. Steyn NP, McHiza ZJ. Obesity and the nutrition transition in Sub-Saharan Africa. *Ann N Y Acad Sci* 2014;**1311:** 88-101.

38. Eriksson JG, Osmond C, Kajantie E, Forsen TJ, Barker DJ. Patterns of growth among children who later develop type 2 diabetes or its risk factors. *Diabetologia* 2006;**49:** 2853-2858.

39. Brisbois TD, Farmer AP, McCargar LJ. Early markers of adult obesity: a review. *Obes Rev* 2012;**13:** 347-367.

40. Corvalán C, Gregory CO, Ramirez-Zea M, Martorell R, Stein AD. Size at birth, infant, early and later childhood growth and adult body composition: a prospective study in a stunted population. *Int J Epidemiol* 2007;**36:** 550-557.

41. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med* 2008;**359:** 61-73.

42. Cooper C, Fall C, Egger P, Hobbs R, Eastell R, Barker D. Growth in infancy and bone mass in later life. *Annals of the rheumatic diseases* 1997;**56:** 17-21.

43. Druet C, Stettler N, Sharp S, Simmons RK, Cooper C, Smith GD*, et al.* Prediction of childhood obesity by infancy weight gain: an individual-level meta-analysis. *Paediatric and perinatal epidemiology* 2012;**26:** 19-26.

44. Barker DJ, Osmond C, Kajantie E, Eriksson JG. Growth and chronic disease: findings in the Helsinki Birth Cohort. *Ann Hum Biol* 2009;**36:** 445-458.

45. Demerath EW, Johnson W, Davern BA, Anderson CG, Shenberger JS, Misra S*, et al.* New body composition reference charts for preterm infants. *The American journal of clinical nutrition* 2017;**105:** 70-77.

Table 1 Overall characteristics for 510 Ethiopian infants and mothers from the iABC cohort included in latent class analyses. Data are presented as mean±SD or % (n)*.

Characteristics	N	mean±SD or % (n)
Birthweight (kg)	507	3.04 ±0.41
Birth fat mass (kg)	507	0.22 ±0.16
Birth fat-free mass (kg)	507	2.82 ±0.34
Birth fat percentage (%)	507	6.85 ±4.63
Birth length (cm)	507	49.1 ±1.9
Low birth weight (%)*	507	9.1% (46)
Maternal age at delivery	499	24.2 ±4.5
Maternal body mass index at delivery	380	22.6 ±3.0
Maternal fat mass index at delivery	371	6.1 ±2.4
Maternal fat-free mass index at delivery	371	16.6 ±1.6
Gestational age (wks)	507	39.0 ±1.0
Sex (female) *	510	49.2% (251)
Parity*	499	
First born		52.5% (262)
2nd born		25.3% (126)
3rd or more		22.2% (111)
Breastfeeding status at 3 months of age *	437	
Exclusive breastfeeding		29.5% (129)
Predominant breastfeeding		68.4% (299)
Not breastfeeding		2.1% (9)
Maternal education*	505	
No or some primary school		53.5% (270)
Completed primary school		15.5% (78)
Completed secondary school		18.2% (92)
Higher education		12.9% (65)

Table 2 Average class probabilities by latent classes

Latent classes	Mean of posterior probabilities					
	Fat class 1	Fat class 2	Fat class 3	Fat class 4	Fat-free class 1	Fat-free class 2
Fat classes						
Fat class 1: Delayed fat	**0.87**	0.11	0.02	0.00		
Fat class 2: Intermediate fat	0.02	**0.87**	0.01	0.10		
Fat class 3: Catch-up fat	0.01	0.09	**0.89**	0.00		
Fat class 4: Accelerated fat	0.00	0.15	0.00	**0.85**		
Fat-free classes						
Fat-free class 1: Quadratic fat-free					**0.81**	0.19
Fat-free class 2: Linear fat-free					0.21	**0.79**

Table 3 Characteristics for 510 infants and mothers in the iABC cohort by fat and fat-free growth classes

Characteristics	Mean (sd) / Percentage (n)							
	Fat growth classes					Fat-free growth classes		
	Delayed fat (n=27)	Intermediate fat (n=303)	Catch-up fat (n=15)	Accelerated fat (n=165)	P	Linear fat-free (n=228)	Quadratic fat-free (n=282)	P
Birthweight (kg)	2.77 ±0.34	2.99 ±0.43[a]	2.86 ±0.44	3.22 ±0.36[abc]	<0.001	2.92 ±0.46	3.15 ±0.36	<0.001
Birth fat mass (kg)	0.16 ±0.13	0.20 ±0.17	0.12 ±0.14	0.28 ±0.16[abc]	<0.001	0.23 ±0.19	0.22 ±0.15	0.829
Birth fat-free mass (kg)	2.60 ±0.29	2.79 ±0.34[a]	2.73 ±0.39	2.94 ±0.30[abc]	<0.001	2.69 ±0.34	2.93 ±0.29	<0.001
Birth fat percentage (%)	5.6 ±4.3	6.3 ±4.9	4.1 ±4.4	8.6 ±4.2 [abc]	<0.001	7.2 ±5.2	6.7 ±4.4	0.378
Birth length (cm)	48.0 ±1.8	48.9 ±2.1[a]	48.5 ±1.9	49.8 ±1.6[abc]	<0.001	48.6 ±2.1	49.6 ±1.8	<0.001
Maternal age at delivery	24.9 ±4.5	24.2 ±4.7	23.8 ±4.0	24.1 ±4.4	0.867	24.0 ±4.6	24.3 ±4.5	0.357
Gestational age (wks)	39.0 ±0.9	38.9 ±1.0	39.1 ±1.1	39.2 ±1.0[b]	0.061	39.0 ±1.0	39.1 ±1.0	0.619
Maternal body mass index at delivery	22.2 ±2.0	22.6 ±3.2	22.7 ±2.2	22.9 ±3.0	0.747	22.7 ±3.0	22.6 ±3.1	0.641
Maternal fat mass index at delivery	5.5 ±2.1	6.1 ±2.4	6.2 ±2.4	6.3 ±2.5[a]	0.213	6.1 ±2.4	6.1 ±2.4	0.937
Maternal fat-free mass index at delivery	16.7 ±1.4	16.5 ±1.7	16.5 ±1.1	16.6 ±1.6	0.789	16.5 ±1.6	16.6 ±1.5	0.087
Sex (Female)	63.0 (17)	47.9 (145)	60.0 (9)	48.5 (80)	0.386	60.5 (138)	40.1 (113)	<0.001
Parity								
First born	52.0 (13)	50.0 (148)	86.7 (13)[ab]	54.0 (88)[c]	0.021	57.7 (127)	48.4 (135)	0.048
2nd born	24.0 (6)	25.3 (75)	6.7 (1)	27.0 (44)	0.474	20.5 (45)	29.0 (81)	0.067
3rd or more	24.0 (6)	24.7 (73)	6.7 (1)	19.2 (31)	0.312	21.8 (48)	22.6 (63)	0.990
Low birth weight (<2.5kg)	22.2 (6)	10.9 (33)	20.0 (3)	2.4 (4)[abc]	0.005	17.1 (39)	2.5 (7)	<0.001
Breastfeeding status at 3 months of age								
Exclusive breastfeeding	20.8 (5)	27.5 (72)	33.3 (5)	34.6 (47)	0.564	23.4 (46)	34.6 (83)	0.005
Predominant breastfeeding	58.3 (14)	71.4 (187)	60.0 (9)	65.4 (89)	0.327	73.1 (144)	64.6 (155)	0.038
Not breastfeeding	20.8 (5)	1.2 (3)[a]	6.7 (1)	0.0 (0)	<0.001	3.6 (7)	0.8 (2)	0.048
Maternal education								
No or some primary school	64.0 (16)	54.3 (163)	40.0 (6)	51.5 (85)	0.437	53.8 (120)	53.2 (150)	0.967
Completed primary school	8.0 (2)	15.7 (47)	13.3 (2)	16.4 (27)	0.689	16.6 (37)	14.5 (41)	0.674
Completed secondary school	20.0 (5)	18.3 (55)	40.0 (6)[b]	15.8 (26)[c]	0.142	19.7 (44)	17.0 (48)	0.416
Higher education	8.0 (2)	11.7 (35)	6.7 (1)	16.4 (27	0.326	9.9 (22)	15.3 (43)	0.146
Quadratic fat-free classes	29.6 (8)	56.8 (172)[a]	33.3 (5)	58.8 (97)[a]	0.053			

Data are presented as mean±SD or %(n). [a] P , 0.05 vs. Delayed fat; [b] P , 0.05 vs. Intermediate fat; [c] P , 0.05 vs. Catch-up fat. All models testing differences between classes were adjusted for sex and maternal age.

Legends

Figure 1 Flow chart of study participants and measurements.

Figure 2 Trajectories of fat mass from birth to 6 months of age for four latent classes with distinct fat mass accretion patterns. The lines show for each class the predicted means and 95% confidence limits. Numbers and density of data points used in the latent class model are presented below the x-axis.

Figure 3 Trajectories of fat-free mass from birth to 6 months of age for two latent classes with distinct fat-free mass accretion patterns. The lines show for each class the predicted means and 95% confidence limits. Numbers and density of data points used in the latent class model are presented below the x-axis.

Figure 1

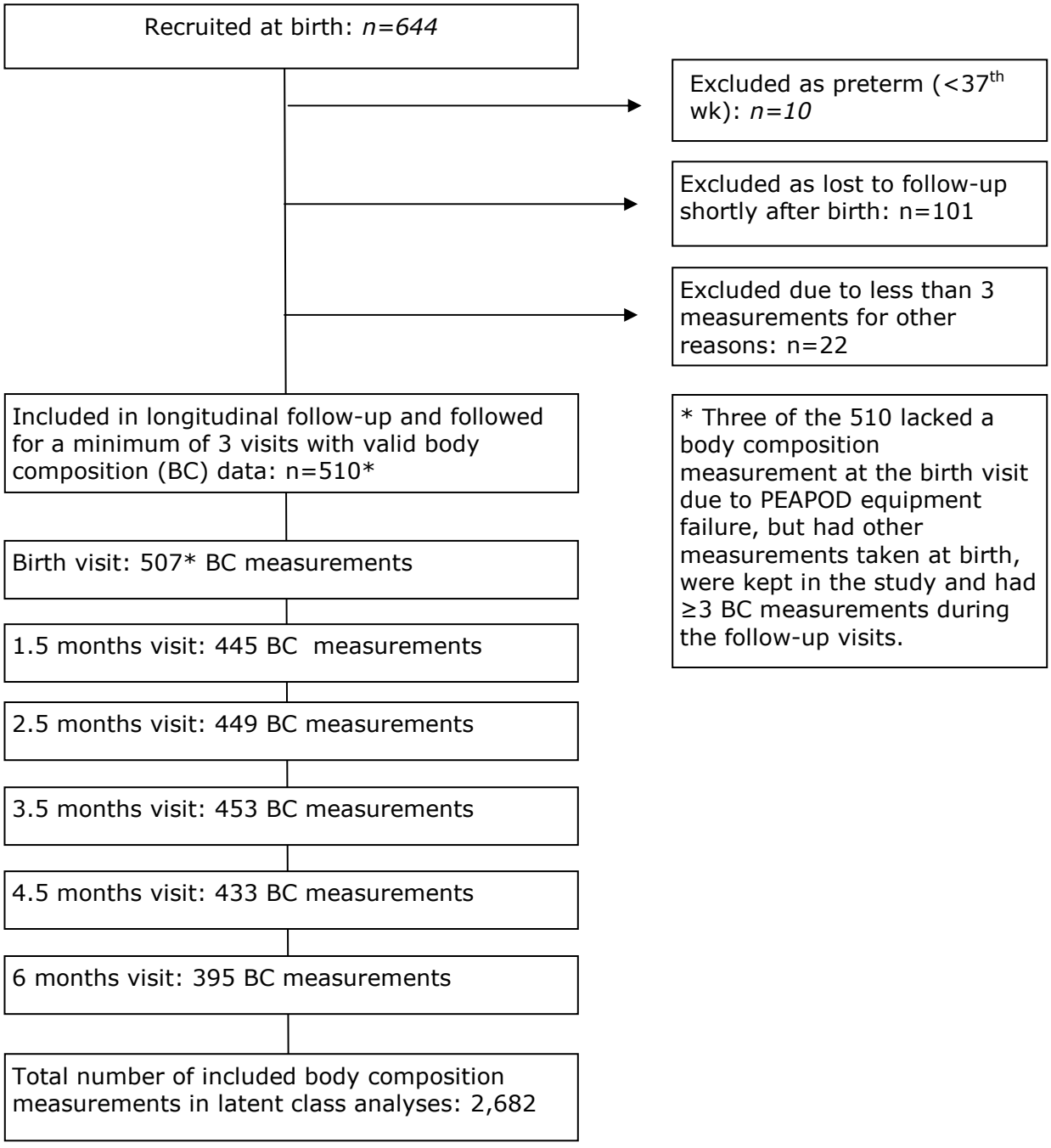

Recruited at birth: *n=644*
Excluded as preterm (<37th wk): *n=10*
Excluded as lost to follow-up shortly after birth: n=101
Excluded due to less than 3 measurements for other reasons: n=22
Included in longitudinal follow-up and followed for a minimum of 3 visits with valid body composition (BC) data: n=510*
* Three of the 510 lacked a body composition measurement at the birth visit due to PEAPOD equipment failure, but had other measurements taken at birth, were kept in the study and had ≥3 BC measurements during the follow-up visits.
Birth visit: 507* BC measurements
1.5 months visit: 445 BC measurements
2.5 months visit: 449 BC measurements
3.5 months visit: 453 BC measurements
4.5 months visit: 433 BC measurements
6 months visit: 395 BC measurements
Total number of included body composition measurements in latent class analyses: 2,682

Figure 2

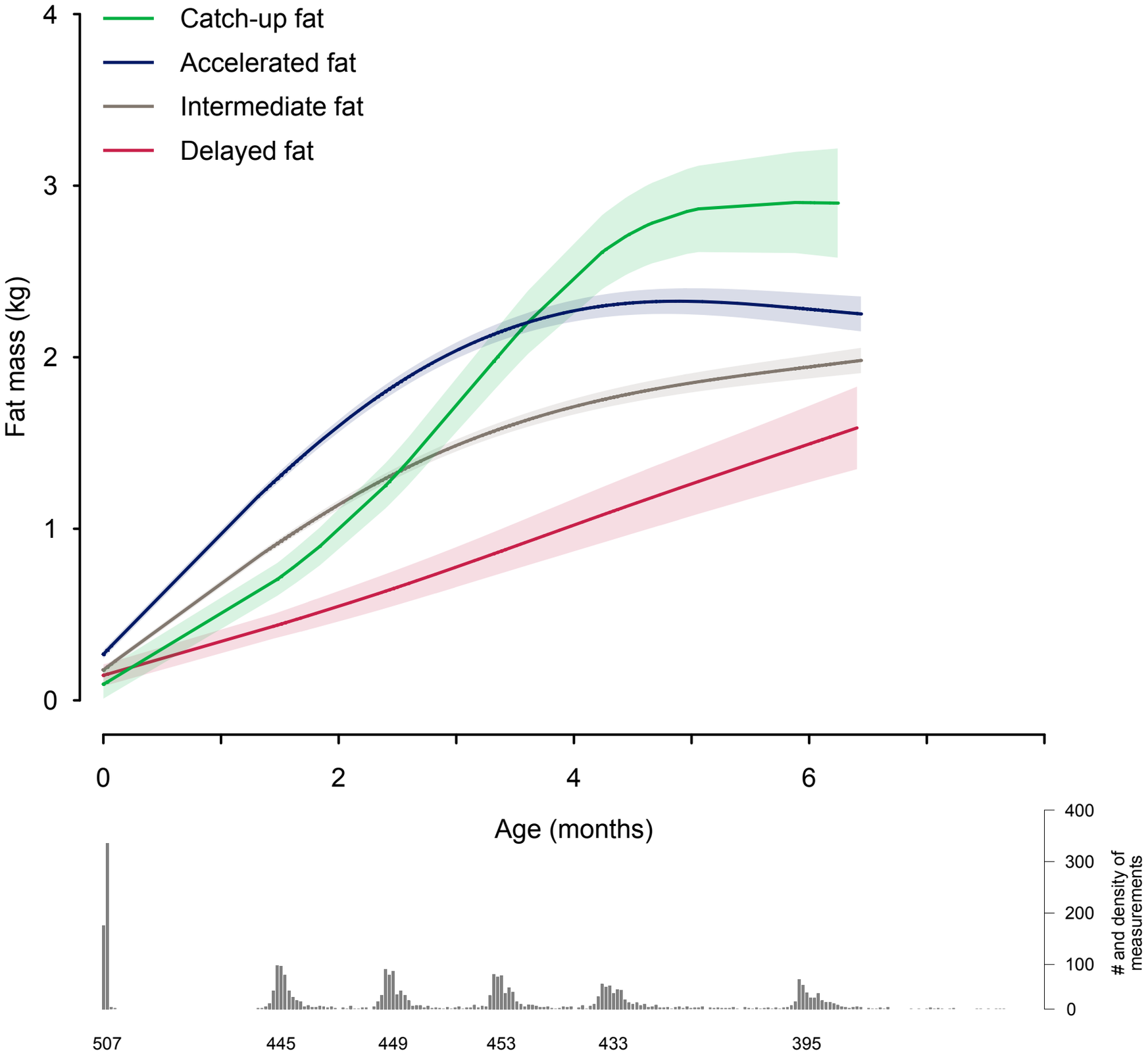
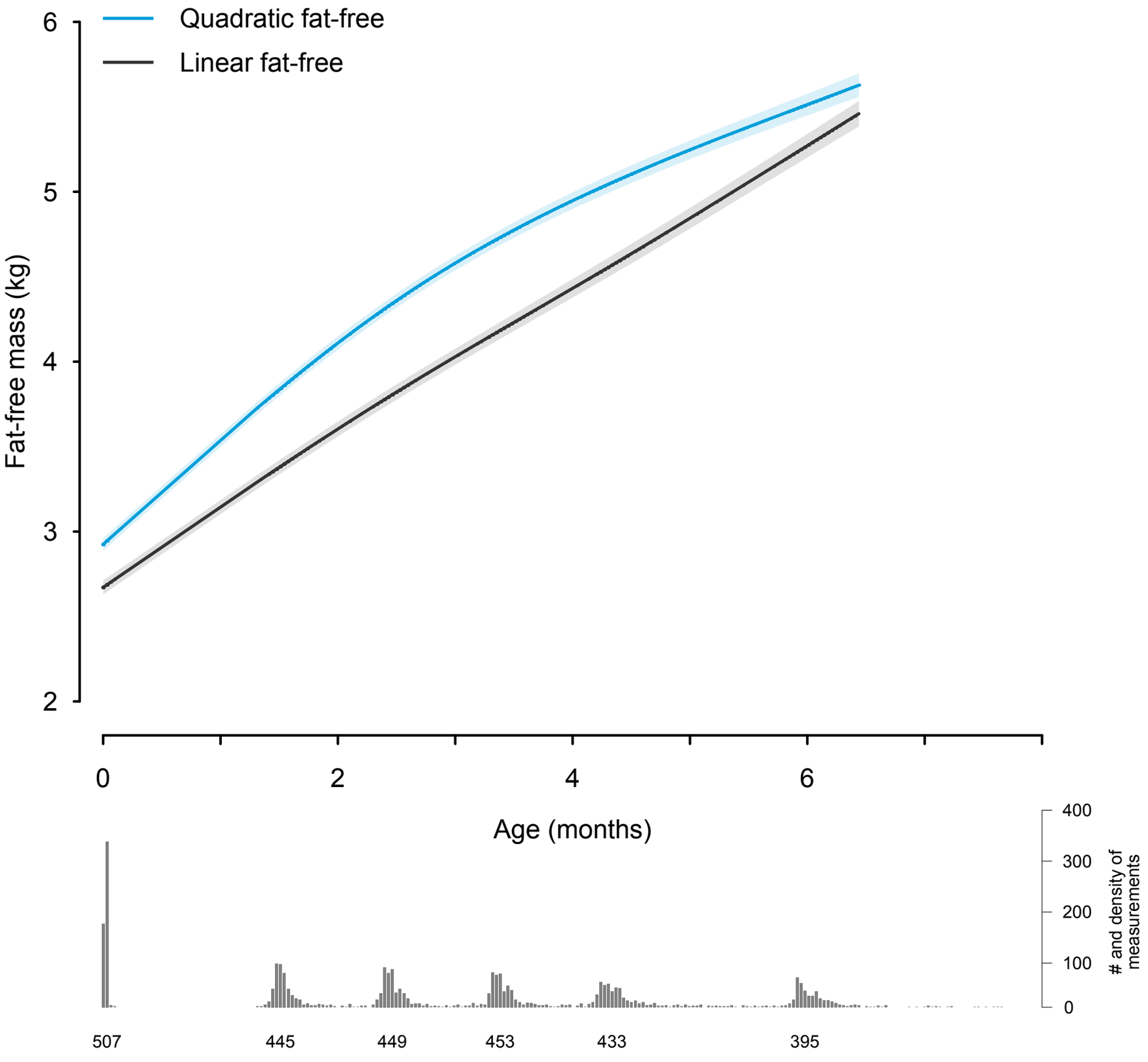

Catch-up fat
Accelerated fat
Intermediate fat
Delayed fat
Fat mass (kg)
0
1
2
3
4
0
2
4
6
Age (months)
400
300
200
100
0
and density of measurements
507
445
449
453
433
395

Figure 3


Quadratic fat-free
Linear fat-free
Fat-free mass (kg)
6
5
4
3
2
0
2
4
6
Age (months)
400
300
200
100
0
and density of measurements
507
445
449
453
433
395

TITLE: Body Composition Growth Patterns in Early Infancy: A Latent Class Trajectory Analysis of the Ethiopian iABC Birth Cohort

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Supporting Information

Detailed description of the latent class trajectory models

The latent class trajectory models were specified as linear mixed-effects models with fat or fat-free mass as the dependent variables with an infant-specific random intercept and slope to account for any potential correlation between repeated measurements within the same infant. The identified latent classes will have different regression parameters for the time variable age.

Age was specified with natural cubic splines with four knots, i.e. cubic polynomials between two adjacent knots, while modelling of the association beyond the outer two knots was restricted to a linear association. A linear term for age was used to specify the random effects of the model.

The ‘‘hlme’’ function in the ‘‘lcmm’’ package in R version 3.2.3 (The R foundation for Statistical Computing) was used to fit the following model and the *“Ns”* function in the *“Epi”* package was used to construct natural cubic splines (example is for fat mass latent classes):

hlme (fat ~ age + spline1 + spline2 + spline3,

mixture =~ age + spline1 + spline2 + spline3,

random =~ , age,

subject = ‘id’,

ng = 4,

data = datasub)

The number of latent classes to optimally describe data was assessed by comparing the fit of models with different number of latent classes. The Bayesian Information Criterion (BIC) was used to evaluate the models and we selected the model with the lowest BIC. A minimum of 3% of the infants in each latent class was required to assure statistical analyses clinical relevance.

Following the model fit, a posterior probability of membership to each of the identified latent classes was calculated for each infant and the infants were assigned exclusively to the class for which the highest probability was obtained.

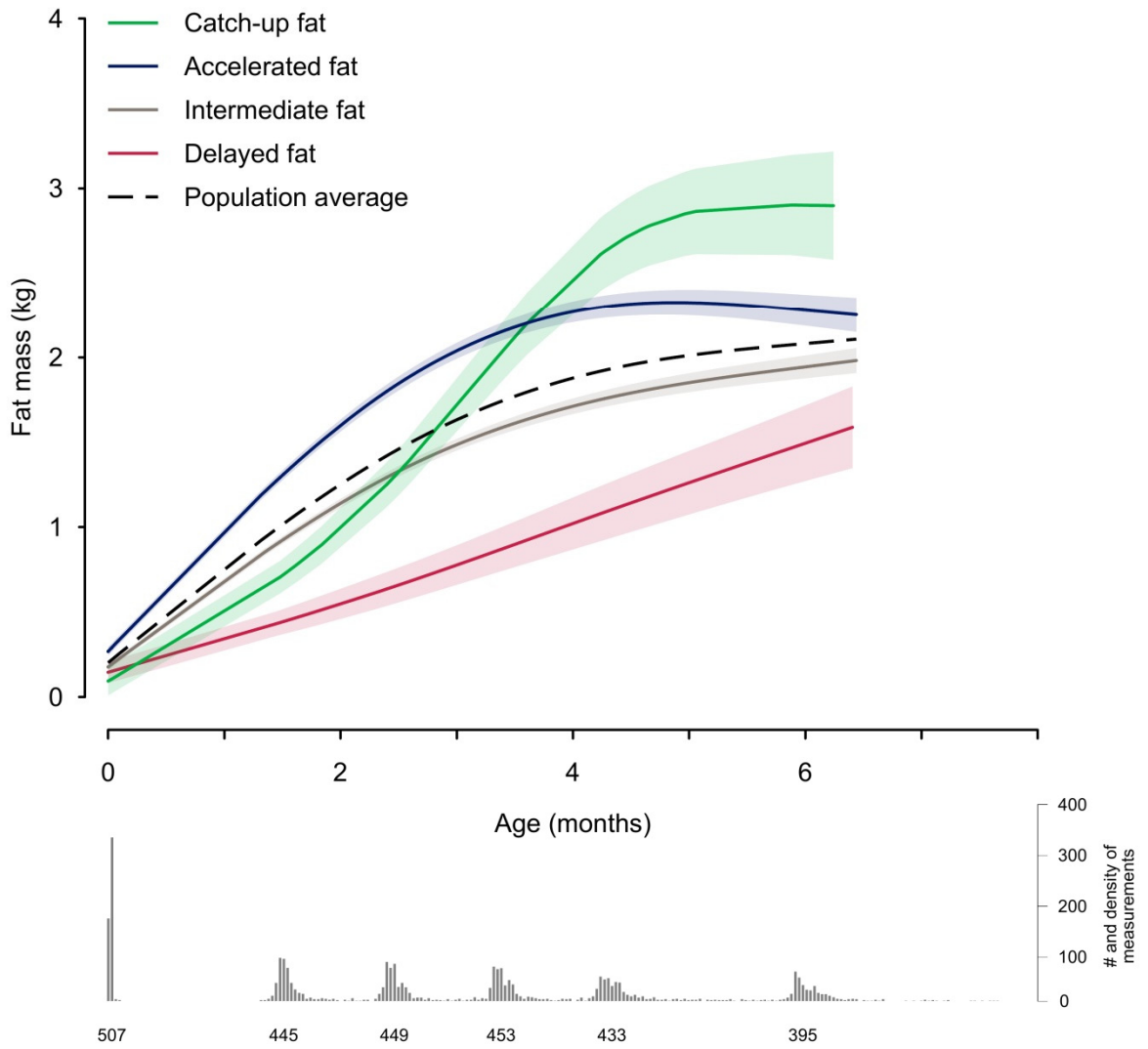

Figure S1 Trajectories of fat mass from birth to 6 months of age for four latent classes with distinct fat mass accretion patterns and for the study population as a whole (black dashed line). The lines show for each class the predicted means and 95% confidence limits, and for the whole population only the predicted mean. Numbers and density of data points used in the latent class model are presented below the x axis.

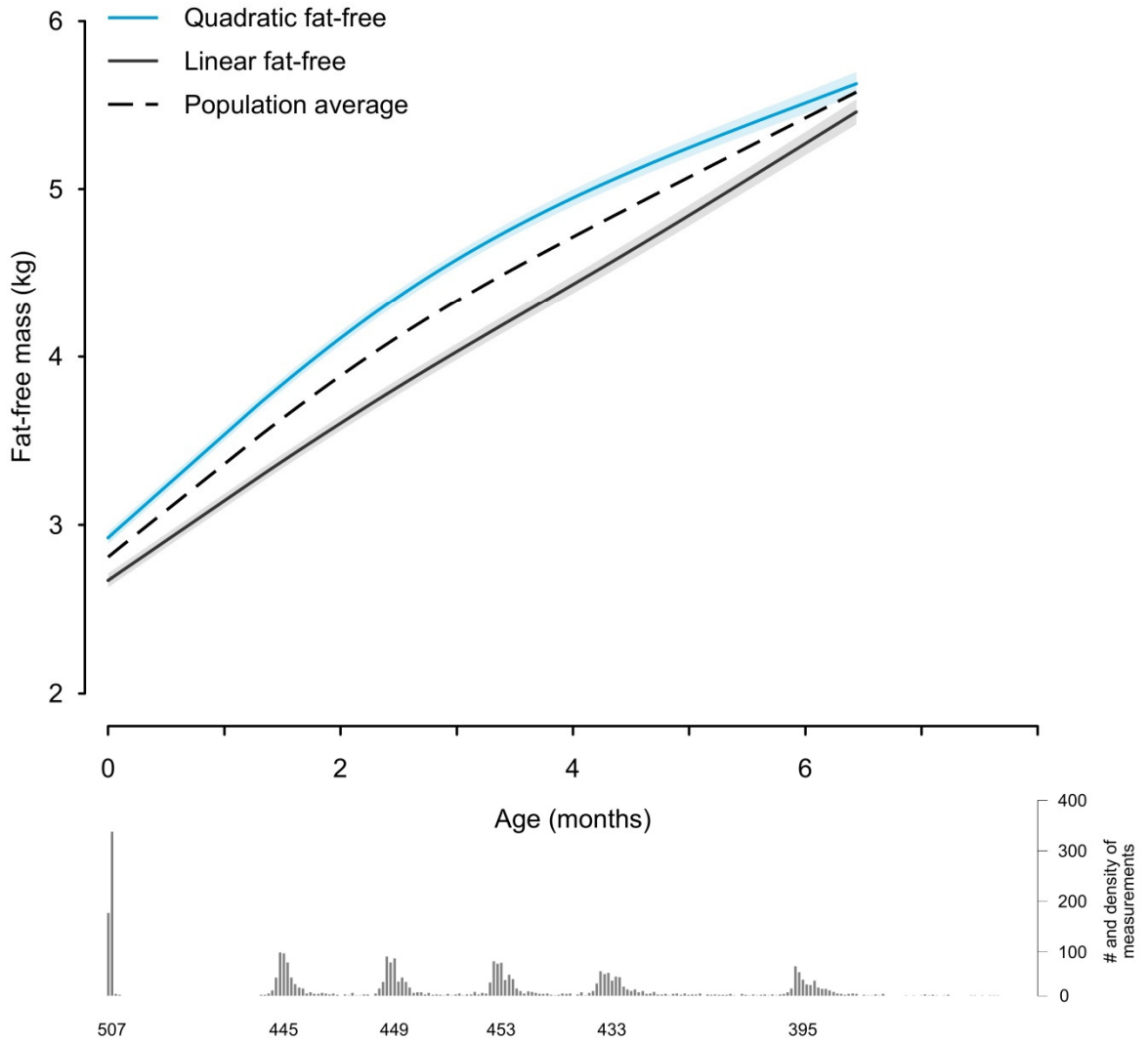

Figure S2 Trajectories of fat-free mass from birth to 6 months of age for two latent classes with distinct fat-free mass accretion patterns and for the study population as a whole (black dashed line). The lines show for each class the predicted means and 95% confidence limits, and for the whole population only the predicted mean. Numbers and density of data points used in the latent class model are presented below the x-axis.

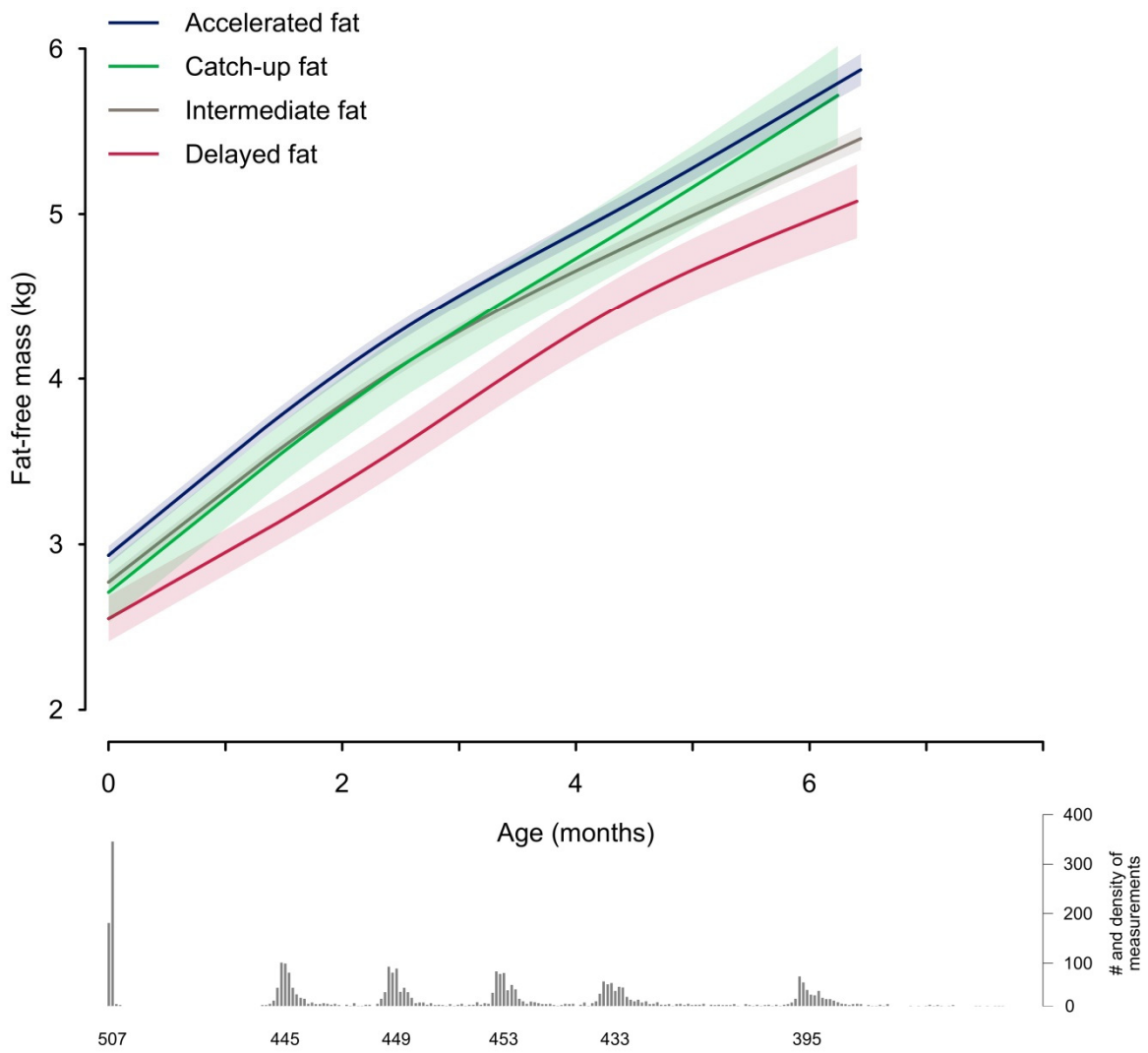

Figure S3 Trajectories of fat-free mass from birth to 6 months of age for four latent classes with distinct fat mass accretion patterns. The lines show for each class the predicted means and 95% confidence limits. Numbers and density of data points used in the latent class model are presented below the x-axis.

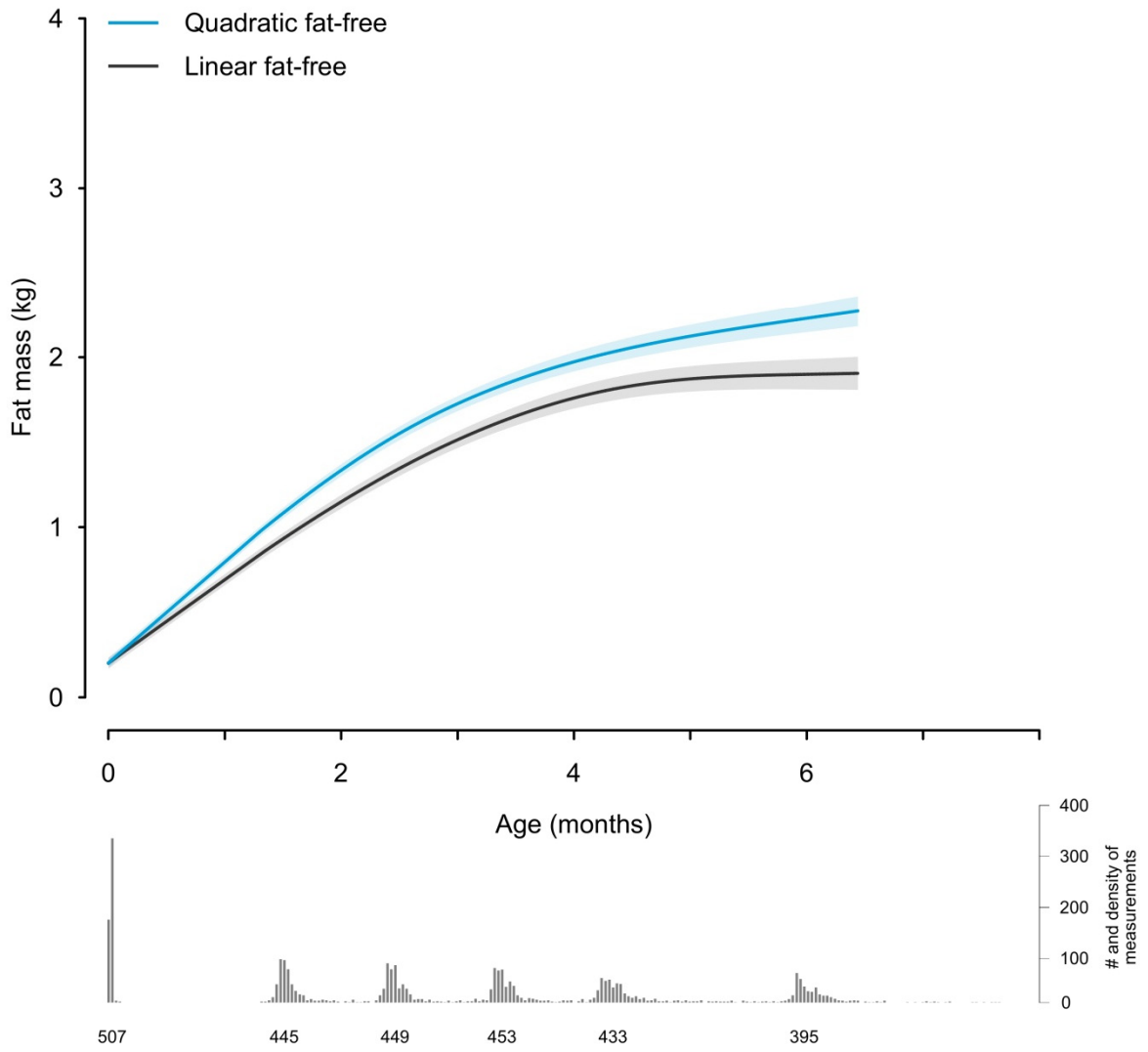

Figure S4 Trajectories of fat mass from birth to 6 months of age for two latent classes with distinct fat-free mass accretion patterns. The lines show for each class the predicted means and 95% confidence limits. Numbers and density of data points used in the latent class model are presented below the x-axis.