Cord blood leptin level and a common variant of its receptor as determinants of the BMI trajectory: The EDEN mother–child cohort

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Title:

Cord blood leptin level and a common variant of its receptor as determinants of the BMI trajectory: the EDEN mother–child cohort

Running title: Cord leptin as a determinant of peak and rebound

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Abbreviations list

AP: Adiposity peak

AR: Adiposity rebound

BMI: Body mass index

EDEN: Etude des Déterminants pré et post natals de la santé de l’Enfant

LEPR: Leptin receptor

SNP: Single nucleotide polymorphism
Background: Cord blood leptin is an indicator of neonatal fat mass and could shape postnatal adiposity trajectories. Investigating genetic polymorphisms of the leptin receptor gene (LEPR) could help understand the mechanisms involved.

Objectives: We aimed to investigate the association of cord blood leptin level and the LEPR rs9436303 polymorphism, with BMI at adiposity peak (AP) and age at adiposity rebound (AR).

Methods: In the EDEN cohort, BMI at AP and age at AR were estimated with polynomial mixed models, for 1713 and 1415 children, respectively. Multivariable linear regression models allowed for examining the associations of cord blood leptin level and LEPR rs9436303 genotype with BMI at AP and age at AR adjusted for potential confounders including birth size groups. We also tested interactions between cord blood leptin level and rs9436303 genotype.

Results: Increased leptin level was associated with reduced BMI at AP and early age at AR (comparing the highest quintile of leptin level to the others). Rs9436303 G-allele carriage was associated with increased BMI at AP and later age at AR but did not modulate the association with leptin level.

Conclusion: These results illustrate the role of early life body composition and the intrauterine environment in the programming of adiposity in childhood.

Keywords: Cord blood leptin, SNP rs9436303, BMI at adiposity peak, age at adiposity rebound, EDEN cohort.
1. Introduction

The body mass index (BMI) trajectory during infancy and childhood is one main predictor of later cardio-metabolic health (1,2) and comprises two key periods. Over the first year of life, the BMI increases to a maximum, called adiposity peak (AP), at about 9 months of age. It then decreases to a minimum and starts to increase again between 4 and 6 years of age, on average, which corresponds to the adiposity rebound (AR) period (3,4). In epidemiological study, higher BMI at AP and earlier age at AR seem to be relevant predictors of high-risk trajectories to later obesity (5–7). These factors are affected by child sex, parental educational level, genetic susceptibility to obesity and parental BMI (8–11). We also previously showed that being born small for gestational age (SGA) and higher maternal gestational weight gain during pregnancy were additional predictors of age at AR (12). These latter findings highlight the important role of intrauterine life in postnatal growth and these prenatal conditions are likely involved in the fetal programming of the adiposity trajectory.

Hormones such as leptin are probably involved in this programming phenomenon and measuring their levels in cord blood may help predict later adiposity trajectories (13,14). Leptin is an adipokine secreted mainly by adipocytes but also by enterocytes in the small intestine and several tissues such as placenta (14). The placenta is one of the main sites for leptin production during pregnancy, and part of the leptin produced in the placenta passes into the fetal circulation. As a consequence, cord blood leptin consists of leptin secreted by fetal adipocytes and placenta but in proportions still unknown (15). Children born SGA exhibit a low level of leptin in cord blood, a low percentage of body fat and higher catch-up growth than others (16–18). Leptin measured in cord blood is therefore considered a marker of neonatal fat mass and is possibly involved in early postnatal growth (19,20). In addition, studies have shown that placental leptin production is increased in severe conditions
associated with fetal growth abnormalities, such as diabetes and pre-eclampsia (21,22).

However, to our knowledge, no study has investigated the role of leptin in the trajectory of BMI, particularly at AP and AR.

To regulate satiety, leptin binds centrally to its hypothalamic receptors and activates multiple signaling pathways that control food intake (23). Mutations in the genes encoding leptin or its receptor (LEPR) can induce leptin resistance and disrupt its central and peripheral actions (23). Some very rare mutations are responsible for severe obesity syndrome in humans (24), and more frequent variants have been associated with BMI variability and obesity in children and adolescents (Aris et al., 2019; Fonseca et al., 2021; Péneau et al., 2017)(23,25). However, the association between individual LEPR variants and the evolution of child BMI has been investigated in few studies and has been suggested to vary according to age (26,27). We hypothesized that cord blood leptin level and carriage of the rare G allele of a common LEPR variant, the rs9436303 single nucleotide polymorphism (SNP), can affect, independently or in interaction, early postnatal growth, and later adiposity trajectories.

To obtain further insights into the role of cord blood leptin level in the development of adiposity in childhood, we aimed to 1) determine whether the cord blood leptin level and the LEPR rs9436303 variant are associated with later BMI trajectory, specifically BMI at AP and age at AR, and 2) examine whether LEPR rs9436303 modulates the association between cord blood leptin level and its indicators of child BMI trajectory.
2. Participants and methods

2.1. Study design

The EDEN (Etude des Déterminants pré et post nataux de la santé de l’ENfant) mother–child study is a prospective French cohort that aims to assess prenatal and postnatal determinants of child growth, health, and development. This cohort recruited 2002 pregnant women (before 24 weeks’ amenorrhea) older than 18 years old between 2003 to 2006 in two university hospital centers in Nancy and Poitiers. All details of the study protocol were published previously (28). Briefly, the exclusion criteria were pre-pregnancy diabetes, multiple pregnancies, inability to read and write in French and planning to move outside the region; 53% of mothers were eligible.

Both parents gave their written consent when their child was included. The Data Protection Authority and the relevant ethical research committee of Kremlin-Bicêtre Hospital gave their approval for the study.

2.2. Cord blood leptin measurement

Immediately after birth, venous cord blood was sampled from 1425 newborns and allowed to clot. Blood samples were centrifuged within 24 h post-collection, serum was collected and stored at -80°C. Cord blood serum was assessed for leptin level using the electro-chemiluminescence (ECL)-based immunoassay V-PLEX Human Leptin Kit (Meso Scale Diagnostics, Rockville, MD, USA). All samples were above the lower limit of detection (LLOD) of 43 pmol/mL. Inter-run coefficients of variations (CV) were below 20%, therefore complying with the recommendations of the US Food and Drug Administration regarding ligand binding assays (29). All assays were completed within 1 week by the same investigator. All assays were performed according to the manufacturer’s instructions.
2.3. Genotyping of LEPR SNP rs9436303

The SNP rs9436303 was previously studied in relation to adiposity peak and age at rebound in the Alves et al. study, in which the EDEN cohort was used for replication (11). Therefore, this polymorphism was the only LEPR SNP genotyped in EDEN and available for this study.

DNA was extracted from cord blood samples. Individual SNP genotyping involved using amplified DNA with TaqMan probes (Applied Biosystems, Foster City, CA, USA). SNP rs9436303 probes were ordered from Applied Biosystems, and after synthesis, used according to the manufacturer’s guidelines. The TaqMan assays were then read on a 7900HT Fast Real-Time PCR System (Applied Biosystems), and the alleles were called by using the SDS software (Applied Biosystems). The call rate was > 98% and the Hardy Weinberg Equilibrium P-value was 0.22. These genetic data were available for 1340 children in the cohort. The G allele of this variant is the rare allele. In this study, we considered the SNP variable (classified as 0, 1, and 2 according to the number of G alleles carried) as a continuous trait, assuming a co-dominant effect on the adiposity traits.

2.4. AP and AR assessments

Children’s weight and height were measured during clinical examinations or collected from child’s health booklets from birth to early adolescence. Weight was measured by previously trained midwives at birth, 1, 3, and 5 years by using an electronic scale (Seca Ltd or Terraillon SL-351). Length was measured at birth and 1 year with a somatometer (Testut, NM Medical), and height at 3 and 5 years with a stadiometer (Seca Ltd). Children had a mean of 10 weight and height measurements (interquartile range 6-14 and 5-13, respectively) from birth to 13 years.
The method used to model individual BMI trajectories, described elsewhere (1), was based on that of Sovio et al. and extended by adding a random effect to each parameter of a mixed-effects cubic model (equation provided in supplementary files). Anthropometric data collected between age 3 days and 24 months were used to determine AP and those between age 18 months and 13 years to determine AR. For each period and each participant, we estimated AP by the first derivative null and second negative derivatives of individual BMI functions and AR by the first derivative null and second positive derivatives of individual BMI function. The modelling was performed over 2 different age periods (between age 3 days and 24 months and between age 18 months and 13 years). The only criterion for inclusion was to have at least three BMI measurements per child over each period. Individual BMI curves were modelled for girls and boys separately for both periods.

As a result, AP data were available for 1713 children and AR data for 1415 children of the cohort. We then considered these two distinct populations according to the outcome of interest (Figure 1).

2.5. Covariates

Maternal smoking status during pregnancy (yes/no), maternal and paternal educational level (years) were collected during pregnancy. At birth, we collected maternal age (years); newborn characteristics including sex; prematurity (yes/no); and birth weight-for-gestational age separated into three classes: SGA (≤ 10th percentile), appropriate for gestational age (AGA, >10th percentile to ≤ 90th percentile), and large for gestational age (LGA, >90th percentile) by using a customized approach calculated according to Gardosi et al. references (30). Gestational weight gain (kg) and gestational diabetes (yes/no) were collected from obstetrics records. Maternal pre-pregnancy weight and paternal weight and height were collected from self-reports at inclusion. Maternal height was measured at inclusion. These measurements
were used to calculate BMI (kg/m²). For simplicity maternal pre-pregnancy BMI and paternal BMI were referred to as parental BMI in the following parts of the manuscript.

2.6. Statistical analysis

Descriptive analyses of leptin level, LEPR SNP and covariates were performed before multiple imputations, with mean (SD) or percentage (N). We compared children with estimated BMI at AP and age at AR to non-included children by Student t test for continuous variables and chi-square test for categorical variables. We also described the mean cord blood leptin level according to rs9436303 genotype and birth size groups.

We used multivariable linear regression models to evaluate the associations of the cord blood leptin level and the rs9436303 genotype with BMI at AP and age at AR. These multivariable linear regression models allow for assessing the association between an exposure of interest (leptin) and our outcome (BMI at AP or age at AR), whatever the level of the other covariates introduced in the models, including birth size group. Analyses were performed separately for each outcome. We did not stratify the analyses by sex because we did not detect a significant interaction between sex and leptin (p>0.3). Given the skewed distribution for the leptin variable, it was log-transformed and standardized by reduced centered mean before being introduced into the models. We first analyzed the unadjusted associations between leptin level, the LEPR SNP and outcomes. We then produced models adjusted for confounders (center, child sex, prematurity, birth size groups, maternal age at delivery, gestational weight gain, gestational diabetes, parental educational level, and parental BMI) for leptin level and the LEPR SNP separately. Finally, we considered leptin level and the LEPR SNP simultaneously in one model adjusted for all confounders. Multivariable analyses used quintiles of cord blood leptin level to assess whether the association was linear.
To test whether the association between leptin level, the LEPR SNP and outcomes did not depend on other variables, we performed two interaction analyses: one between cord blood leptin level and rs9436303 genotype and another between cord blood leptin level and birth size groups.

We used multiple imputation (SAS MI and MIANALYZE procedures) techniques to deal with missing values due to leptin level, the LEPR SNP and covariates (Supplementary table S1) to reduce potential selection bias. We generated 40 independent imputed datasets using the fully conditional specification method. For each dataset, we used inverse probability weighting (IPW) to account for potential attrition bias. We assigned to each participant a weight corresponding to the inverse probability of being included in the analysis sample in a logistic regression model adjusted for covariates (31). The results of different imputed datasets were combined by using the SAS MIANALYZE procedure, in which standard errors are calculated using Rubin’s rules (32).

As sensitivity analyses, we repeated all analyses on complete cases (children without missing values for covariates) and after excluding mothers with pre-eclampsia because this condition has been associated with elevated placental leptin secretion (21).

We used R v3.5.1 for modelling BMI trajectories and estimating AP and AR (package NLME). SAS v9.4 (SAS, Cary, NC, on an AIX 7.1 platform) was used for all other analyses. Nominal p <0.05 was considered statistically significant.
3. Results

3.1. Characteristics of study populations

The children included in our two samples (children with estimated BMI at AP and children with estimated age at AR) were in general similar (Table 1). The mean [SD] BMI at AP was 17.5 [1.3] kg/m² and mean age at AR 5.5 [1.4] years. As compared with children excluded from sample 1, children with estimated BMI at AP, children included had a higher mean cord blood leptin level and the proportion of preterm births was lower (Supplementary Table S2). The mothers of the children included in the two samples had a higher level of education, were older, and were less frequently smokers during pregnancy than those of the excluded children. We found no difference in distribution of the LEPR variant. The proportion of children with each variant of the LEPR SNP was similar between children included or not in both samples (p=0.53 and 0.80 for samples 1 and 2, respectively). The allelic frequency of the G allele was 26.1% and 26.6% in samples 1 and 2, respectively.

Infants born SGA had a lower mean [SD] cord blood leptin level than those with AGA and LGA (8.3 [0.99] vs 8.9 [0.84] and 9.5 [0.85], p<.0001) (Supplementary figure S1a). We found no significant difference between mean cord blood leptin level according to number of risk alleles (Supplementary figure S1b).

3.2. Cord blood leptin level, BMI at AP, and age at AR

Cord blood leptin level was negatively related to BMI at AP in both the unadjusted (mean [SE] = -0.14 [0.04] kg/m²; p=0.0001) and the fully adjusted (-0.19 [0.04] kg/m²; p<.0001) models (Table 2). Furthermore, investigating quintiles of cord blood leptin revealed a linear shape of the association with BMI at AP (p trend <.0001) (Figure 2A).

The negative association between cord blood leptin level and age at AR before any adjustment (Table 2) did not remain significant in the multivariable model. However, when
considering quintiles of leptin level in the model (Figure 2B), the highest quintile was negatively associated with age at AR as compared with all the other quintiles combined (mean [SE] = -87.3 [39.4] days or about 2.9 [1.3] months; p=0.03).

After adjusting for cord blood leptin level, as compared with children born AGA, those born SGA had lower BMI at AP (-0.57 [0.10] kg/m²; p<.0001) and earlier age at AR (-101.0 [39.8] days or about -3.2 [1.3] months; p=0.01), whereas those born LGA had higher BMI at AP (0.54 [0.12] kg/m²; p<.0001) (Tables 2). We did not find any significant interaction between birth size groups and cord blood leptin level (p>0.15).

3.3. LEPR polymorphism, BMI at AP, and age at AR
In the fully adjusted model, carrying a rare allele (G) in the LEPR SNP was associated with increased BMI at AP by about 0.24 kg/m² (p<0.001) and delayed age at AR by about 67 days (2.2 months; p=0.005) (Tables 2). We did not find any interaction between leptin level and rs9436303 G-allele carriage with the two outcomes considered (p-interaction=0.48 and 0.28 for BMI at AP and age at AR, respectively).

3.4. Sensitivity analyses
The results of the complete-case analyses are presented in Supplementary table S3. Results from analyses restricted to children with no missing data were consistent overall with those after multiple imputation and IPW. When mother–child pairs presenting pre-eclampsia pregnancy complications were excluded from the analysis, the results remained unchanged (data not shown).
4. Discussion

In the prospective EDEN mother–child cohort, we demonstrated that whatever the birth size group, increased cord blood leptin level was associated with reduced BMI at AP and earlier age at AR when considering the highest quintile of leptin level versus the other quintiles (Figure 2). Carrying the rare G-allele of the LEPR SNP rs9436303 did not modulate these associations but was independently related to higher BMI at AP and later age at AR.

High BMI at AP and early age at AR have been associated with increased BMI, risk of cardiovascular disease, and obesity later in childhood and in adulthood (1–3). However, in their work on the ELANCE cohort, Rolland-Cachera and Péneau highlighted two trajectories at risk of later obesity (5): a first one marked by a high BMI at any age and a second characterized by low BMI at AP followed by an early rebound and a subsequent increase in BMI. According to this study, children with early AR had lower BMI before and higher BMI after the rebound, on average, and were at increased risk of overweight in adulthood (5). We interpreted our results on the association between cord blood leptin and the LEPR polymorphism in light of these previously described trajectory.

To our knowledge, no previous studies have investigated cord blood leptin in association with such specific features but rather analyzed BMI at specific ages in infancy or childhood. In a prospective study of 642 children born in Greece, Karakosta et al. showed high cord blood leptin level related to reduced BMI from age 6 months to 4 years (33). Likewise, other studies of mother–infant pairs reported a significant inverse association between cord blood leptin level and both BMI standard deviation score and weight gain in the first year of life (34–36). Several analyses conducted in the project VIVA cohort showed low cord blood leptin level related to small birth size (14) and increased BMI z-score at age 3.
years (13) but low measures of adiposity from childhood to early adolescence (37). A recent meta-analysis including some of these studies concluded that higher cord blood leptin levels were associated with lower adiposity up to 3 years of age but not between 4 and 7 years (38). Our results are fully consistent with this overview because we show high cord blood leptin level associated with reduced BMI at AP but earlier age at AR, recognized as a risk factor for later obesity (1,2,6,7). Furthermore, this trajectory, characterized by a reduced BMI at AP more likely followed by early age at the time of AR (dashed line in Supplementary Figure S2), corresponds precisely to one of the two risk trajectories described by Rolland-Cachera and Peneau (5).

Mechanisms underlying such associations are still unclear but deserve to be elucidated and discussed. The leptin hormone regulates satiety, and its circulating level is positively correlated with body fat mass and negatively correlated with satiety (39–41). Reduced circulating leptin level enhances food intake and increases body fat mass and vice versa (19,39). This is consistent with our finding showing lower leptin level at birth associated with higher BMI at AP. Furthermore, animal studies suggest that in addition to its role in satiety, leptin would contribute to the modification of the release of other growth hormones in the hypothalamus (42) and to the regulation of certain neuroendocrine functions (43).

Our findings could explain in part the rapid postnatal weight gain observed in children born SGA as compared with other children (44,45). Indeed, the level of leptin was lower, on average, in children born SGA than their peers. However, in our study, being born SGA and high cord blood leptin level were independent predictors of reduced BMI at AP, which suggests that different physiological mechanisms are involved in catch-up growth. Indeed, infants are born SGA usually due to poor fetal growth and nutrition. Children born SGA
exhibit abnormal body composition with lower lean mass and at higher risk for metabolic disorders (46,47).

Our results also reveal that very high cord blood leptin level was associated with early age at AR. Leptin measured in venous cord blood consists of placental and fetal leptin (19,48). The placenta is one of the main sites for leptin production during pregnancy, and part of the leptin produced in the placenta passes into the fetal circulation, but the proportion of placental leptin concentration measured in cord blood at birth is unknown (15). Thus, cord blood leptin reflects both placental and fetal leptin. According to some studies, placental leptin production is increased during fetal hypoxia in response to an adverse intrauterine condition (21,49). Cord blood leptin level higher than expected for a given amount of fat mass could be a marker of adverse intrauterine conditions, themselves associated with BMI trajectories characterized by early AR (46,47). This suggestion is consistent with the hypothesis of the existence of a fetal programming for BMI trajectory at the time of AR suggested by several authors (50–53). Our sensitivity analysis revealed similar results with or without the exclusion of mothers with pre-eclampsia, but the number of pre-eclampsia mothers was very low.

To exert its action on satiety in the individual, leptin binds to hypothalamic receptors. Altered LEPR gene expression may lead to leptin resistance and thus modulate the satiety effect of leptin during childhood and affect the BMI trajectory and AR. Leptin resistance is characterized by the inability of leptin to reach the target cell owing to reduced expression of LEPR or disrupted LEPR signaling. This resistance leads to decreased effectiveness of its action and a major decrease in the effect of leptin on satiety (54). Thus, the effects of leptin on appetite and body mass are impaired and may result in higher leptin secretion as a compensatory mechanism. Its action on satiety occurs in the postnatal period only, so there is
no reason to believe that a change in \textit{LEPR} level could affect prenatal leptin production as a compensatory mechanism, which explains why no association between leptin levels in cord blood and \textit{LEPR} genotype was expected. Indeed, we did not find any difference in cord blood leptin level between carriers or not of the \textit{LEPR} \texttt{rs9436303} rare G-allele. Our hypothesis that the G allele would interact in the association between cord blood leptin level and early growth was not confirmed by our results. However, children carrying the G allele had higher BMI at AP and later age at AR (dotted line in \textit{Supplementary figure S2}). In a previous genome-wide association study, which included 7215 children from five European cohorts, carrying the G allele was also associated with higher BMI at AP, and the \texttt{rs9436303} genotype explained 0.3\% of the variance in BMI at AP (11) as compared with 1.3\% in our study (data not shown). To our knowledge, the association between \texttt{rs9436303} genotype and age at AR, has not been investigated in genetic studies. Another study addressed the link between 28 \textit{LEPR} polymorphisms and BMI in 522 Spanish children aged 6 to 15 years and showed a strong association between \texttt{rs11804091} carriage and obesity-related traits in children(23). The \texttt{rs11804091} rare allele frequency was found in linkage disequilibrium with \texttt{rs9436303} (R$^2$=0.502). Therefore, variants in \textit{LEPR} could determine the overall BMI trajectory, regardless of cord blood leptin level, and future studies should analyze the long-term association with adult obesity. A systematic analysis and meta-analysis of 17 studies in adults examined the association between three other variants of \textit{LEPR} and obesity-related phenotypes and did not find any association with adult overweight (55). Genetic variation in \textit{LEPR} may have different effects depending on the variant considered and the age window explored, as with leptin level. Further studies are needed to better understand the physiological mechanisms involved and the long-term consequences of \textit{LEPR} genetic variation on the BMI trajectory.
This study is among the first to examine the links of both cord blood leptin level and of the LEPR SNP rs9436303 genotype with BMI trajectory, especially at the times of AP and AR. Anthropometric data were collected prospectively, which allowed us to estimate AP and AR for many children in the EDEN cohort. However, the generalization of results remains limited because of potential selection and attrition biases that we attempted to address by applying multiple imputation and IPW. The complete-case analyses showed well that the impact of these biases was limited. Moreover, cord blood leptin reflects both placental and fetal leptin, which limits interpretation regarding the origin of leptin variation (placenta, fetal) in our sample. Of note, previous studies have shown that cord blood leptin level was not correlated with maternal leptin level or BMI but with birth weight and length, ponderal index, and weight/length ratio (20,56). These data support that cord blood leptin reflects fetal fat mass, which suggests that leptin measured in cord blood is primarily of fetal origin and strengthens the relevance of relying on cord blood leptin level as a proxy of neonatal adiposity. However, our study was not designed with the aim of developing a biological signature at birth, that could serve as a tool in routine clinical practice for the early detection of children at risk of developing obesity during childhood; further dedicated studies would be necessary for this purpose.

In conclusion, we have shown that cord blood leptin level and LEPR SNP rs9436303 genotype interfere with BMI trajectory in children, independent of each other and birth size group. These results illustrate the role of early life body composition in the programming of childhood adiposity. In addition to being a marker of fetal adiposity, cord blood leptin level could also reflect an unfavorable intrauterine environment and may predict a trajectory at risk for subsequent obesity.
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Author contributions

None of the authors have any financial relationships or conflict of interest to disclose.

AC and BH designed the research, wrote the manuscript and analyzed the data.

BH, MAC, MuT, MaT, SL, KL, OK, LD and BLG were responsible for data collection and genotyping in EDEN. All authors reviewed drafts, provided critical feedback, approved the final manuscript and were responsible for the final content of the paper. BH had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflict of interest

The authors declare that they have no conflict of interest.
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Figure 1: Flow chart of the children included in the study. Two separate samples based on the outcomes with adiposity peak on the left and adiposity rebound on the right. BMI, body mass index; SNP, single nucleotide polymorphism.
Figure 2: Adjusted means\(^a\) (SE) for A) BMI at adiposity peak (AP) and B) age at adiposity rebound (AR) according to cord leptin level quintiles\(^b\).

\(^a\) Adjusted for center, maternal age, maternal and paternal education, maternal and paternal BMI, gestational weight gain, gestational diabetes, maternal smoking during pregnancy, prematurity, birth size groups and sex.

\(^b\) Quintiles 1, 2, 3 and 4 were grouped together and compared to the last quintile in the model.
Table 1: Description of study populations

|                                | Children with estimated BMI at AP N=1713 | Children with estimated age at AR N=1415 |
|--------------------------------|-----------------------------------------|------------------------------------------|
|                                | Mean (SD) or % (N)                       | Mean (SD) or % (N)                       |
| Sex (boys)                     | 51.8 (888)                              | 52.3 (746)                               |
| Preterm birth (yes)            | 5.4 (92)                                | 5.5 (78)                                 |
| Birth weight (kg)              | 3.3 (5.1)                               | 3.3 (5.0)                                |
| Birth size groups               |                                         |                                          |
| SGA                            | 13.1 (218)                              | 13.3 (184)                               |
| AGA                            | 78.4 (1309)                             | 77.4 (1070)                              |
| LGA                            | 8.6 (143)                               | 9.3 (128)                                |
| Gestational weight gain (kg)   | 13.4 (4.7)                              | 13.3 (4.7)                               |
| Gestational diabetes (yes)     | 6.65 (94)                               | 6.60 (113)                               |
| Maternal BMI (kg/m²)           | 23.2 (4.6)                              | 23.1 (4.4)                               |
| Paternal BMI (kg/m²)           | 25.1 (3.7)                              | 25.1 (3.6)                               |
| Never smoking during pregnancy | 75.8 (1269)                             | 78.6 (1088)                              |
| Maternal age at delivery (years) | 29.7 (4.8)                              | 29.9 (4.7)                               |
| Maternal educational level (years) | 13.7 (2.6)                              | 14.0 (2.6)                               |
| Paternal educational level (years) | 13.1 (2.6)                              | 13.3 (2.6)                               |
| Age at AR (years)              | 5.5 (1.4)                               | 5.5 (1.4)                                |
| BMI at AP (kg/m²)              | 17.5 (1.3)                              | 17.5 (1.3)                               |
| Cord blood leptin level (ng/ml) | 11.1 (11.0)                             | 11.0 (10.5)                              |
| LEPR SNP rs9436303             |                                         |                                          |
| A/A                            | 55.4 (668)                              | 55.0 (551)                               |
| A/G                            | 36.9 (445)                              | 36.8 (369)                               |
| G/G                            | 7.6 (92)                                | 8.2 (82)                                 |
| G-allele frequency             | 26.1                                    | 26.6                                     |

* Small for gestational age, appropriate for gestational age and large for gestational age classified according to Gardosi references.
AP, adiposity peak; AR, adiposity rebound; BMI, body mass index; SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; SNP, single nucleotide polymorphism
Table 2: Results of association analyses between cord blood leptin level, LEPR SNP rs9436303 and BMI at AP (in kg/m², N=1713\(^a\)) and age at AR (in days, N=1415\(^b\))

| Characteristics | Separate and unadjusted models \(^c\) | Separate adjusted model with leptin level \(^d\) | Separate adjusted model with LEPR SNP \(^d\) | Fully adjusted model \(^d\) |
|-----------------|------------------------------------------|-----------------------------------------------|-----------------------------------------------|-------------------------------|
|                 | \(\beta\) (SE) \(p\ value\)         | \(\beta\) (SE) \(p\ value\)               | \(\beta\) (SE) \(p\ value\)               | \(B\) (SE) \(p\ value\)     |
| **Outcome = BMI at AP; N=1713\(^a\)** | | | | |
| Birth size groups \(^e\) (ref=AGA) | | | | |
| SGA             | -0.57 (0.10) <.0001                  | -0.48 (0.10) <.0001                       | -0.57 (0.10) <.0001                       | -0.57 (0.10) <.0001         |
| LGA             | 0.54 (0.12) <.0001                  | 0.43 (0.12) 0.0002                       | 0.54 (0.12) <.0001                       | -0.19 (0.04) <.0001         |
| Cord blood leptin (log-transformed) | -0.14 (0.04) 0.0001                | -0.19 (0.04) <.0001                       | 0.24 (0.06) <.0001                       | 0.24 (0.06) <.0001         |
| LEPR SNP rs9436303 (number of G alleles carried) | 0.25 (0.06) <.0001                | 0.24 (0.06) <.0001                       | 0.24 (0.06) <.0001                       | 0.24 (0.06) <.0001         |
| **Outcome = Age at AR; N=1415\(^b\)** | | | | |
| Birth size groups \(^e\) (ref=AGA) | | | | |
| SGA             | -101.0 (39.8) 0.01              | -94.0 (39.0) 0.02                        | -104.5 (39.9) 0.009                      | -104.5 (39.9) 0.009         |
| LGA             | 20.9 (47.9) 0.66               | 5.6 (46.8) 0.91                         | 18.1 (47.8) 0.70                         | 18.1 (47.8) 0.70            |
| Cord blood leptin (log-transformed) | -34.9 (15.0) 0.02               | -24.7 (17.6) 0.16                       | -22.7 (17.6) 0.20                       | -22.7 (17.6) 0.20          |
| LEPR SNP rs9436303 (number of G alleles carried) | 65.3 (24.5) 0.008               | 67.9 (23.6) 0.004                       | 66.6 (23.7) 0.005                       | 66.6 (23.7) 0.005          |

\(^a\) Sample of children with estimated BMI at AP, linear regression model after multiple imputation on missing data of leptin, LEPR SNP and covariates.

\(^b\) Sample of children with estimated age at AR, linear regression model after multiple imputation on missing data of leptin, LEPR SNP and covariates.

\(^c\) Two separate models for cord blood leptin and rs9436303 without any adjustment.

\(^d\) Adjusted for center, maternal age, maternal and paternal education, maternal and paternal BMI, gestational weight gain, gestational diabetes, maternal smoking during pregnancy, prematurity and sex.

\(^e\) Small for gestational age, appropriate for gestational age and large for gestational age classified according to Gardosi references.
Supplementary Materials and Methods: Modeling equation for BMI trajectories

The following equation describes the general model applied for the $i^{th}$ individual at the $t^{th}$ time point, whatever the period considered (12).

$$\log(\text{BMI}_{it}) = \beta_0 + \sum_{j=1}^{3} \beta_j \text{Age}_{it}^j + b_{0i} + \sum_{j=1}^{3} b_{ji} \text{Age}_{it}^j + \epsilon_{it}$$

Where:

- $\beta_0, \beta_1, \beta_2, \beta_3$ are the parameters for the fixed effects,
- $b_{0i}, b_{1i}, b_{2i}, b_{3i}$ are the parameter estimates for the random effects on the polynomial coefficients, for each individual,
- $\epsilon_{it}$ are the error terms assumed to be normally distributed and independent
### Supplementary Table S1: Details of the method used for multiple imputation

| Variables                                | Type of variable | Model used to predict missing data | Missing value (%) |
|------------------------------------------|------------------|------------------------------------|-------------------|
| Age at adiposity peak                    | Continuous       |                                    | 0                 |
| BMI at adiposity peak                    | Continuous       |                                    | 0                 |
| Age at adiposity rebound                 | Continuous       |                                    | 0                 |
| BMI at adiposity rebound                 | Continuous       |                                    | 0                 |
| Center                                   | Binary           |                                    | 0                 |
| Sex                                      | Binary           |                                    | 0                 |
| Birth weight                             | Continuous       | Linear regression                  | 0.06              |
| Personalized birth weight z-score        | Continuous       | Linear regression                  | 2.5               |
| Height at birth                          | Continuous       | Linear regression                  | 2.9               |
| Head circumference at birth              | Continuous       | Linear regression                  | 2.9               |
| Skin folds at birth                      | Continuous       | Linear regression                  | 5.8               |
| Gestational age                          | Continuous       |                                    | 0                 |
| Weight predicted at 5 years by Jenss-Bayley modelling | Continuous |                                    | 0                 |
| Height predicted at 5 years by Jenss-Bayley modelling | Continuous |                                    | 0                 |
| Maternal age at delivery                 | Continuous       |                                    | 0                 |
| Gestational weight gain                  | Continuous       | Linear regression                  | 1.9               |
| Gestational diabetes                     | Binary           | Logistic regression                 | 0.6               |
| Smoking during pregnancy                 | Binary           | Logistic regression                 | 2.3               |
| Maternal education                       | Continuous       |                                    | 0                 |
| Maternal income                          | Ordinal (3 categories) | Multinomial regression | 0.6               |
| Maternal BMI                             | Continuous       | Linear regression                  | 2.0               |
| Paternal education                       | Continuous       |                                    | 0                 |
| Paternal BMI                             | Continuous       | Linear regression                  | 8.0               |
| Cord blood leptin level                  | Continuous       | Linear regression                  | 25.0              |
| rs9436303 SNP                            | Ordinal (3 categories) | Multinomial regression | 29.7              |

From the variables in the table, we generated 40 independent sets of imputed data using the fully conditional specification method (MI procedure of the SAS software). This method assigned data to missing measurements based on the measurement of infants with similar profiles. Then, we computed pooled effect estimates (MIANALYZE procedure of the SAS software).

BMI, body mass index; SNP, single nucleotide polymorphism

a Fully conditional specification method.

b Predicted weight and height were calculated by using previously modelled trajectories from the Jenss–Bayley model (1, 2).

1. Carles, S. et al. A Novel Method to Describe Early Offspring Body Mass Index (BMI) Trajectories and to Study Its Determinants. *PLOS ONE* **11**, e0157766 (2016).
2. Botton, J. et al. Postnatal Weight and Height Growth Modeling and Prediction of Body Mass Index as a Function of Time for the Study of Growth Determinants. *Ann. Nutr. Metab.* **65**, 156–166 (2014)
### Table S2: Characteristics of children included or not in each analysis sample

| Characteristics                     | Children with estimated BMI at AP | Children with estimated age at AR |
|-------------------------------------|-----------------------------------|----------------------------------|
|                                     | Included N=1713 Excluded N=289 | Included N=1415 Excluded N=587   |
|                                     | Mean (SD) or % (N)               | P > |t| or χ²                          |
| Sex (boys)                          | 51.8 (888) 59.0 (112)           | 0.06                             |
|                                     |                                  |                                  |
| Preterm birth (yes)                 | 5.4 (92) 9.4 (18)                | 0.02                             |
|                                     |                                  |                                  |
| Birth weight (kg)                   | 3.3 (0.5) 3.2 (0.5)              | 0.13                             |
|                                     |                                  |                                  |
| Birth size groups a                  |                                  | 0.38                             |
| SGA                                 | 13.1 (218) 15.3 (28)             | 0.38                             |
| AGA                                 | 78.4 (1309) 78.7 (144)           | 0.38                             |
| LGA                                 | 8.6 (143) 6.0 (11)               | 0.38                             |
| Gestational weight gain (kg)        | 13.4 (4.7) 13.7 (5.7)            | 0.41                             |
| Gestational diabetes (yes)          | 6.65 (94) 5.92 (29)              | 0.57                             |
| Maternal BMI (kg/m²)                | 23.2 (4.6) 23.5 (5.2)            | 0.43                             |
| Paternal BMI (kg/m²)                | 25.1 (3.7) 24.4 (3.4)            | 0.02                             |
| Never smoking during pregnancy      | 75.8 (1269) 54.0 (94)            | <.0001                           |
| Maternal age at delivery (years)    | 29.7 (4.8) 27.9 (5.4)            | <.0001                           |
| Maternal educational level (years)  | 13.7 (2.6) 12.3 (2.5)            | <.0001                           |
| Paternal educational level (years)  | 13.1 (2.6) 12.2 (2.2)            | <.0001                           |
Compari

age at AR to non-included children from eligible mothers by Student t test for continuous variables and chi-square test for categorical variable

a Small for gestational age, appropriate for gestational age and large for gestational age classified according to Gardosi references.

BMI, body mass index; AP, adiposity peak; AR, adiposity rebound, SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; SNP, single nucleotide polymorphism

|                  |                |                |                |                |                |
|------------------|----------------|----------------|----------------|----------------|----------------|
| Age at AR (years)| 5.5 (1.4)      |                |                |                |                |
| BMI at AP (kg/m²)| 17.5 (1.34)    |                |                |                |                |
| Cord blood leptin level (ng/ml) | 11.1 (11.0) | 9.3 (9.2) | 0.04 | 11.0 (10.5) | 10.8 (11.9) | 0.78 |
| LEPR SNP rs9436303 |                | 0.53 |                |                |                |
| A/A              | 55.4 (668)     | 54.1 (73)      | 55.0 (551)     | 56.2 (190)     |                |
| A/G              | 36.9 (445)     | 35.6 (48)      | 36.8 (369)     | 36.7 (124)     |                |
| G/G              | 7.6 (92)       | 10.4 (14)      | 8.2 (82)       | 7.1 (24)       |                |
Supplementary Table S3: Results of association analyses of complete cases between cord blood leptin level, *LEPR* SNP rs9436303 SNP and BMI at AP and age at AR

| Characteristics                                      | Separate and unadjusted models<sup>a</sup> | Separate adjusted model with leptin level<sup>b</sup> | Separate adjusted model with *LEPR* SNP<sup>b</sup> | Fully adjusted model<sup>b</sup> |
|------------------------------------------------------|--------------------------------------------|--------------------------------------------------------|---------------------------------------------------|---------------------------------|
| **Outcome = BMI at AP**                               |                                            | N=1124                                                 | N=1047                                            | N=948                           |
| Birth size groups<sup>c</sup> (ref=AGA)               |                                            | β (SE) p value                                         | β (SE) p value                                    | B (SE) p value                   |
| SGA                                                  |                                            | -0.54 (0.13) <.0001                                   | -0.48 (0.13) 0.0002                               | -0.46 (0.14) 0.001               |
| LGA                                                  |                                            | 0.63 (0.14) <.0001                                    | 0.42 (0.14) 0.003                                 | 0.59 (0.15) <.0001               |
| Cord blood leptin (log-transformed)                  |                                            | -0.26 (0.04) 0.0002                                   | -0.21 (0.04) <.0001                               | -0.20 (0.05) <.0001              |
| *LEPR* SNP rs9436303 (number of G alleles carried)    |                                            | 0.24 (0.06) <.0001                                   | 0.22 (0.07) 0.001                                 | 0.24 (0.07) 0.001                |
| **Outcome = Age at AR**                              |                                            | N=928                                                  | N=880                                             | N=793                           |
| Birth size groups<sup>c</sup> (ref=AGA)               |                                            |                                                        |                                                   |                                 |
| SGA                                                  |                                            | -172.46 (52.16) 0.001                                 | -163.13 (53.38) 0.002                             | -181.82 (56.90) 0.002            |
| LGA                                                  |                                            | 37.12 (56.12) 0.51                                    | 3.05 (56.11) 0.96                                | 30.75 (59.72) 0.61              |
| Cord blood leptin (log-transformed)                  |                                            | -26.94 (15.34) 0.08                                   | -28.44 (18.42) 0.12                               | -25.40 (19.43) 0.19             |
| *LEPR* SNP rs9436303 (number of G alleles carried)    |                                            | 57.84 (24.50) 0.02                                    | 80.04 (26.16) 0.002                               | 85.00 (27.46) 0.002             |

<sup>a</sup> Two separate models for cord blood leptin and rs9436303 without any adjustment.

<sup>b</sup> Adjusted for center, maternal age, maternal and paternal education, maternal and paternal BMI, gestational weight gain, gestational diabetes, maternal smoking during pregnancy, prematurity and sex.

<sup>c</sup> Small for gestational age, appropriate for gestational age and large for gestational age classified according to Gardosi references.
Supplementary Figure S1: Violin plots with quantile bounds and means [95% confidence interval]. Cord blood leptin level by A) birth size groups and B) LEPR SNP rs9436303 G-allele carriage. The dotted lines represent the quantiles (from bottom to top: 10th, 25th, 75th and 90th percentiles). The symmetrical curves represent the probability densities at different values of leptin. SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age.
Supplementary Figure S2: *Three fictitious BMI trajectories illustrating the results obtained in this analysis. Dotted line represents higher BMI at AP and later rebound; trajectory associated with carrying a rare G-allele of the rs9436303 SNP. Dashed line represents lower BMI at AP followed by earlier age at AR, trajectory associated with an increase in the cord blood leptin level; trajectory associated with carrying a rare G-allele of the rs9436303 SNP. And solid line a trajectory with normal BMI at AP and age at AR.*