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

Aminata Cissé, Marion Taine, Muriel Tafflet, Blandine de Lauzon-Guillain, Karine Clément, Olfa Khalfallah, Laetitia Davidovic, Sandrine Lioret, Marie Charles, Barbara Heude

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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

1 **Abstract (200/200)**

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

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

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

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

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

20 **Keywords:** Cord blood leptin, SNP rs9436303, BMI at adiposity peak, age at adiposity  
21 rebound, EDEN cohort.

## 22        **1. Introduction**

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

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

46 associated with fetal growth abnormalities, such as diabetes and pre-eclampsia (21,22).  
47 However, to our knowledge, no study has investigated the role of leptin in the trajectory of  
48 BMI, particularly at AP and AR.

49 To regulate satiety, leptin binds centrally to its hypothalamic receptors and activates  
50 multiple signaling pathways that control food intake (23). Mutations in the genes encoding  
51 leptin or its receptor (*LEPR*) can induce leptin resistance and disrupt its central and peripheral  
52 actions (23). Some very rare mutations are responsible for severe obesity syndrome in  
53 humans (24), and more frequent variants have been associated with BMI variability and  
54 obesity in children and adolescents (Aris et al., 2019; Fonseca et al., 2021; Péneau et al.,  
55 2017)(23,25). However, the association between individual *LEPR* variants and the evolution  
56 of child BMI has been investigated in few studies and has been suggested to vary according to  
57 age (26,27). We hypothesized that cord blood leptin level and carriage of the rare G allele of a  
58 common *LEPR* variant, the rs9436303 single nucleotide polymorphism (SNP), can affect,  
59 independently or in interaction, early postnatal growth, and later adiposity trajectories.

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

65 **2. Participants and methods**

66 **2.1. Study design**

67 The EDEN (Etude des Déterminants pré et post natals de la santé de l'ENfant) mother-child  
68 study is a prospective French cohort that aims to assess prenatal and postnatal determinants of  
69 child growth, health, and development. This cohort recruited 2002 pregnant women (before  
70 24 weeks' amenorrhea) older than 18 years old between 2003 to 2006 in two university  
71 hospital centers in Nancy and Poitiers. All details of the study protocol were published  
72 previously (28). Briefly, the exclusion criteria were pre-pregnancy diabetes, multiple  
73 pregnancies, inability to read and write in French and planning to move outside the region;  
74 53% of mothers were eligible.

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

78 **2.2. Cord blood leptin measurement**

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

88                   **2.3. Genotyping of *LEPR* SNP rs9436303**

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

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

102                   **2.4. AP and AR assessments**

103    Children’s weight and height were measured during clinical examinations or collected from  
104    child’s health booklets from birth to early adolescence. Weight was measured by previously  
105    trained midwives at birth, 1, 3, and 5 years by using an electronic scale (SecaLtd or Terraillon  
106    SL-351). Length was measured at birth and 1 year with a somatometer (Testut,NMMedical),  
107    and height at 3 and 5 years with a stadiometer (Seca Ltd). Children had a mean of 10 weight  
108    and height measurements (interquartile range 6- 14 and 5-13, respectively) from birth to 13  
109    years.



110 The method used to model individual BMI trajectories, described elsewhere (1), was  
111 based on that of Sovio *et al.* and extended by adding a random effect to each parameter of a  
112 mixed-effects cubic model (equation provided in supplementary files). Anthropometric data  
113 collected between age 3 days and 24 months were used to determine AP and those between  
114 age 18 months and 13 years to determine AR. For each period and each participant, we  
115 estimated AP by the first derivative null and second negative derivatives of individual BMI  
116 functions and AR by the first derivative null and second positive derivatives of individual  
117 BMI function. The modelling was performed over 2 different age periods (between age 3 days  
118 and 24 months and between age 18 months and 13 years). The only criterion for inclusion was  
119 to have at least three BMI measurements per child over each period. Individual BMI curves  
120 were modelled for girls and boys separately for both periods.

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

## 124 **2.5. Covariates**

125 Maternal smoking status during pregnancy (yes/no), maternal and paternal educational level  
126 (years) were collected during pregnancy. At birth, we collected maternal age (years); newborn  
127 characteristics including sex; prematurity (yes/no); and birth weight-for-gestational age  
128 separated into three classes: SGA ( $\leq 10^{\text{th}}$  percentile), appropriate for gestational age (AGA,  
129  $>10^{\text{th}}$  percentile to  $\leq 90^{\text{th}}$  percentile), and large for gestational age (LGA,  $>90^{\text{th}}$  percentile) by  
130 using a customized approach calculated according to Gardosi *et al.* references (30).  
131 Gestational weight gain (kg) and gestational diabetes (yes/no) were collected from obstetrics  
132 records. Maternal pre-pregnancy weight and paternal weight and height were collected from  
133 self-reports at inclusion. Maternal height was measured at inclusion. These measurements

134 were used to calculate BMI ( $\text{kg}/\text{m}^2$ ). For simplicity maternal pre-pregnancy BMI and paternal  
135 BMI were referred to as parental BMI in the following parts of the manuscript.

## 136 **2.6. Statistical analysis**

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

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

157 To test whether the association between leptin level, the *LEPR* SNP and outcomes did not  
158 depend on other variables, we performed two interaction analyses: one between cord blood  
159 leptin level and rs9436303 genotype and another between cord blood leptin level and birth  
160 size groups.

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

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

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

176 **3. Results**

177 **3.1. Characteristics of study populations**

178 The children included in our two samples (children with estimated BMI at AP and children  
179 with estimated age at AR) were in general similar (Table 1). The mean [SD] BMI at AP was  
180 17.5 [1.3] kg/m<sup>2</sup> and mean age at AR 5.5 [1.4] years. As compared with children excluded  
181 from sample 1, children with estimated BMI at AP, children included had a higher mean cord  
182 blood leptin level and the proportion of preterm births was lower (Supplementary Table S2).  
183 The mothers of the children included in the two samples had a higher level of education, were  
184 older, and were less frequently smokers during pregnancy than those of the excluded children.  
185 We found no difference in distribution of the *LEPR* variant. The proportion of children with  
186 each variant of the *LEPR* SNP was similar between children included or not in both samples  
187 (p=0.53 and 0.80 for samples 1 and 2, respectively). The allelic frequency of the G allele was  
188 26.1% and 26.6% in samples 1 and 2, respectively.

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

193 **3.2. Cord blood leptin level, BMI at AP, and age at AR**

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

198 The negative association between cord blood leptin level and age at AR before any  
199 adjustment (Table 2) did not remain significant in the multivariable model. However, when

200 considering quintiles of leptin level in the model (Figure 2B), the highest quintile was  
201 negatively associated with age at AR as compared with all the other quintiles combined (mean  
202 [SE] = -87.3 [39.4] days or about 2.9 [1.3] months; p=0.03).

203 After adjusting for cord blood leptin level, as compared with children born AGA,  
204 those born SGA had lower BMI at AP (-0.57 [0.10] kg/m<sup>2</sup>; p<.0001) and earlier age at AR (-  
205 101.0 [39.8] days or about -3.2 [1.3] months; p=0.01), whereas those born LGA had higher  
206 BMI at AP (0.54 [0.12] kg/m<sup>2</sup>; p<.0001) (Tables 2). We did not find any significant  
207 interaction between birth size groups and cord blood leptin level (p>0.15).

### 208 **3.3. *LEPR* polymorphism, BMI at AP, and age at AR**

209 In the fully adjusted model, carrying a rare allele (G) in the *LEPR* SNP was associated with  
210 increased BMI at AP by about 0.24 kg/m<sup>2</sup> (p<0.001) and delayed age at AR by about 67 days  
211 (2.2 months; p=0.005) (Tables 2). We did not find any interaction between leptin level and  
212 rs9436303 G-allele carriage with the two outcomes considered (p-interaction=0.48 and 0.28  
213 for BMI at AP and age at AR, respectively).

### 214 **3.4. Sensitivity analyses**

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

#### 220 4. Discussion

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

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

235 To our knowledge, no previous studies have investigated cord blood leptin in  
236 association with such specific features but rather analyzed BMI at specific ages in infancy or  
237 childhood. In a prospective study of 642 children born in Greece, Karakosta *et al.* showed  
238 high cord blood leptin level related to reduced BMI from age 6 months to 4 years (33).  
239 Likewise, other studies of mother–infant pairs reported a significant inverse association  
240 between cord blood leptin level and both BMI standard deviation score and weight gain in the  
241 first year of life (34–36). Several analyses conducted in the project VIVA cohort showed low  
242 cord blood leptin level related to small birth size (14) and increased BMI z-score at age 3

243 years (13) but low measures of adiposity from childhood to early adolescence (37). A recent  
244 meta-analysis including some of these studies concluded that higher cord blood leptin levels  
245 were associated with lower adiposity up to 3 years of age but not between 4 and 7 years (38).  
246 Our results are fully consistent with this overview because we show high cord blood leptin  
247 level associated with reduced BMI at AP but earlier age at AR, recognized as a risk factor for  
248 later obesity (1,2,6,7). Furthermore, this trajectory, characterized by a reduced BMI at AP  
249 more likely followed by early age at the time of AR (dashed line in Supplementary Figure  
250 S2), corresponds precisely to one of the two risk trajectories described by Rolland-Cachera  
251 and Peneau (5).

252         Mechanisms underlying such associations are still unclear but deserve to be elucidated  
253 and discussed. The leptin hormone regulates satiety, and its circulating level is positively  
254 correlated with body fat mass and negatively correlated with satiety (39–41). Reduced  
255 circulating leptin level enhances food intake and increases body fat mass and vice versa  
256 (19,39). This is consistent with our finding showing lower leptin level at birth associated with  
257 higher BMI at AP. Furthermore, animal studies suggest that in addition to its role in satiety,  
258 leptin would contribute to the modification of the release of other growth hormones in the  
259 hypothalamus (42) and to the regulation of certain neuroendocrine functions (43).

260         Our findings could explain in part the rapid postnatal weight gain observed in children  
261 born SGA as compared with other children (44,45). Indeed, the level of leptin was lower, on  
262 average, in children born SGA than their peers. However, in our study, being born SGA and  
263 high cord blood leptin level were independent predictors of reduced BMI at AP, which  
264 suggests that different physiological mechanisms are involved in catch-up growth. Indeed,  
265 infants are born SGA usually due to poor fetal growth and nutrition. Children born SGA

266 exhibit abnormal body composition with lower lean mass and at higher risk for metabolic  
267 disorders (46,47).

268         Our results also reveal that very high cord blood leptin level was associated with early  
269 age at AR. Leptin measured in venous cord blood consists of placental and fetal leptin  
270 (19,48). The placenta is one of the main sites for leptin production during pregnancy, and part  
271 of the leptin produced in the placenta passes into the fetal circulation, but the proportion of  
272 placental leptin concentration measured in cord blood at birth is unknown (15). Thus, cord  
273 blood leptin reflects both placental and fetal leptin. According to some studies, placental  
274 leptin production is increased during fetal hypoxia in response to an adverse intrauterine  
275 condition (21,49). Cord blood leptin level higher than expected for a given amount of fat mass  
276 could be a marker of adverse intrauterine conditions, themselves associated with BMI  
277 trajectories characterized by early AR (46,47). This suggestion is consistent with the  
278 hypothesis of the existence of a fetal programming for BMI trajectory at the time of AR  
279 suggested by several authors (50–53). Our sensitivity analysis revealed similar results with or  
280 without the exclusion of mothers with pre-eclampsia, but the number of pre-eclampsia  
281 mothers was very low.

282         To exert its action on satiety in the individual, leptin binds to hypothalamic receptors.  
283 Altered *LEPR* gene expression may lead to leptin resistance and thus modulate the satiety  
284 effect of leptin during childhood and affect the BMI trajectory and AR. Leptin resistance is  
285 characterized by the inability of leptin to reach the target cell owing to reduced expression of  
286 *LEPR* or disrupted *LEPR* signaling. This resistance leads to decreased effectiveness of its  
287 action and a major decrease in the effect of leptin on satiety (54). Thus, the effects of leptin on  
288 appetite and body mass are impaired and may result in higher leptin secretion as a  
289 compensatory mechanism. Its action on satiety occurs in the postnatal period only, so there is



290 no reason to believe that a change in *LEPR* level could affect prenatal leptin production as a  
291 compensatory mechanism, which explains why no association between leptin levels in cord  
292 blood and *LEPR* genotype was expected. Indeed, we did not find any difference in cord blood  
293 leptin level between carriers or not of the *LEPR* rs9436303 rare G-allele. Our hypothesis that  
294 the G allele would interact in the association between cord blood leptin level and early growth  
295 was not confirmed by our results. However, children carrying the G allele had higher BMI at  
296 AP and later age at AR (dotted line in [Supplementary figure S2](#)). In a previous genome-wide  
297 association study, which included 7215 children from five European cohorts, carrying the G  
298 allele was also associated with higher BMI at AP, and the rs9436303 genotype explained  
299 0.3% of the variance in BMI at AP (11) as compared with 1.3% in our study (data not shown).  
300 To our knowledge, the association between rs9436303 genotype and age at AR, has not been  
301 investigated in genetic studies. Another study addressed the link between 28 *LEPR*  
302 polymorphisms and BMI in 522 Spanish children aged 6 to 15 years and showed a strong  
303 association between rs11804091 carriage and obesity-related traits in children(23). The  
304 rs11804091 rare allele frequency was found in linkage disequilibrium with rs9436303  
305 ( $R^2=0.502$ ). Therefore, variants in *LEPR* could determine the overall BMI trajectory,  
306 regardless of cord blood leptin level, and future studies should analyze the long-term  
307 association with adult obesity. A systematic analysis and meta-analysis of 17 studies in adults  
308 examined the association between three other variants of *LEPR* and obesity-related  
309 phenotypes and did not find any association with adult overweight (55). Genetic variation in  
310 *LEPR* may have different effects depending on the variant considered and the age window  
311 explored, as with leptin level. Further studies are needed to better understand the  
312 physiological mechanisms involved and the long-term consequences of *LEPR* genetic  
313 variation on the BMI trajectory.

314           This study is among the first to examine the links of both cord blood leptin level and  
315 of the *LEPR* SNP rs9436303 genotype with BMI trajectory, especially at the times of AP and  
316 AR. Anthropometric data were collected prospectively, which allowed us to estimate AP and  
317 AR for many children in the EDEN cohort. However, the generalization of results remains  
318 limited because of potential selection and attrition biases that we attempted to address by  
319 applying multiple imputation and IPW. The complete-case analyses showed well that the  
320 impact of these biases was limited. Moreover, cord blood leptin reflects both placental and  
321 fetal leptin, which limits interpretation regarding the origin of leptin variation (placenta, fetal)  
322 in our sample. Of note, previous studies have shown that cord blood leptin level was not  
323 correlated with maternal leptin level or BMI but with birth weight and length, ponderal index,  
324 and weight/length ratio (20,56). These data support that cord blood leptin reflects fetal fat  
325 mass, which suggests that leptin measured in cord blood is primarily of fetal origin and  
326 strengthens the relevance of relying on cord blood leptin level as a proxy of neonatal  
327 adiposity. However, our study was not designed with the aim of developing a biological  
328 signature at birth, that could serve as a tool in routine clinical practice for the early detection  
329 of children at risk of developing obesity during childhood; further dedicated studies would be  
330 necessary for this purpose.

331           In conclusion, we have shown that cord blood leptin level and *LEPR* SNP rs9436303  
332 genotype interfere with BMI trajectory in children, independent of each other and birth size  
333 group. These results illustrate the role of early life body composition in the programming of  
334 childhood adiposity. In addition to being a marker of fetal adiposity, cord blood leptin level  
335 could also reflect an unfavorable intrauterine environment and may predict a trajectory at risk  
336 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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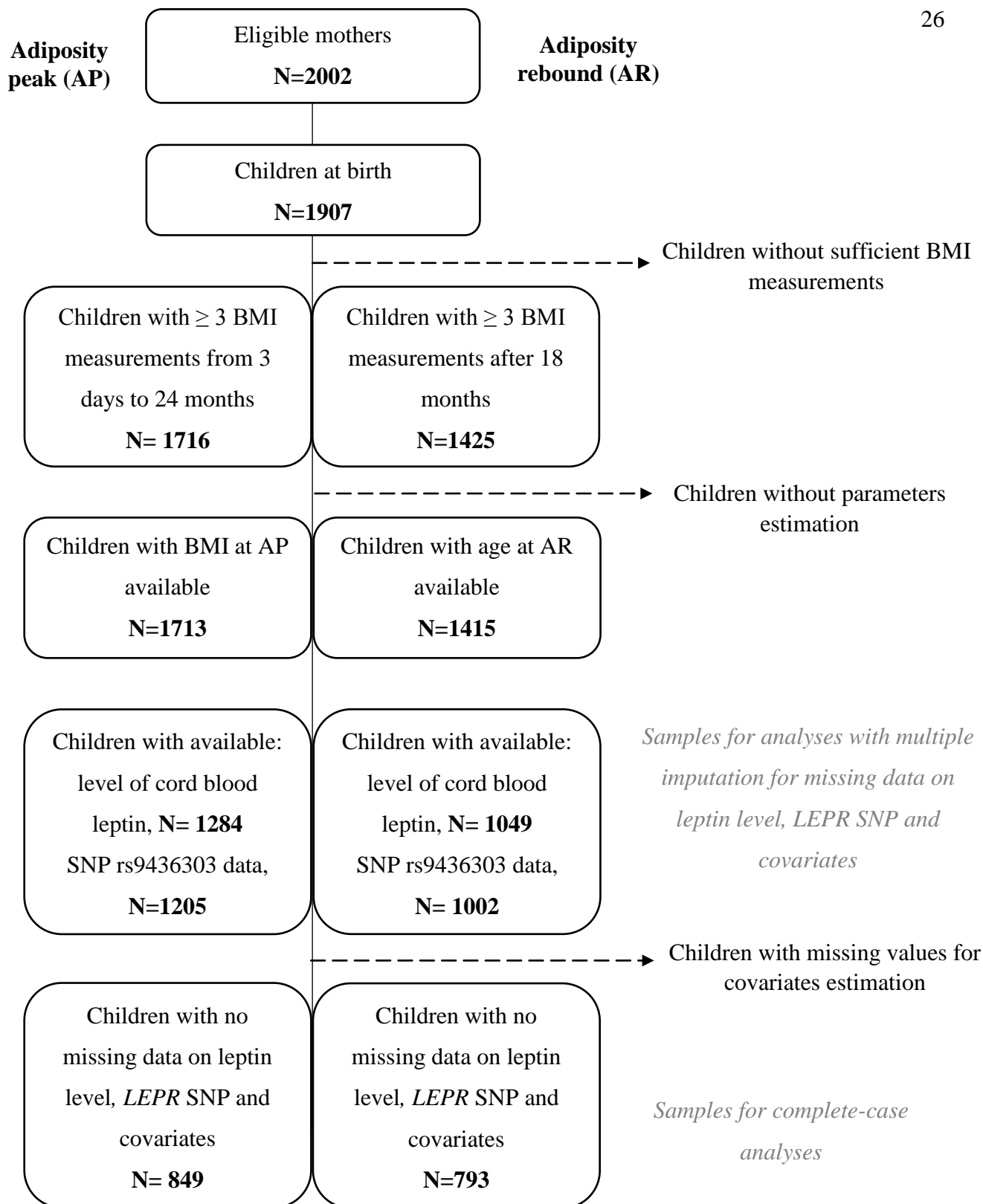
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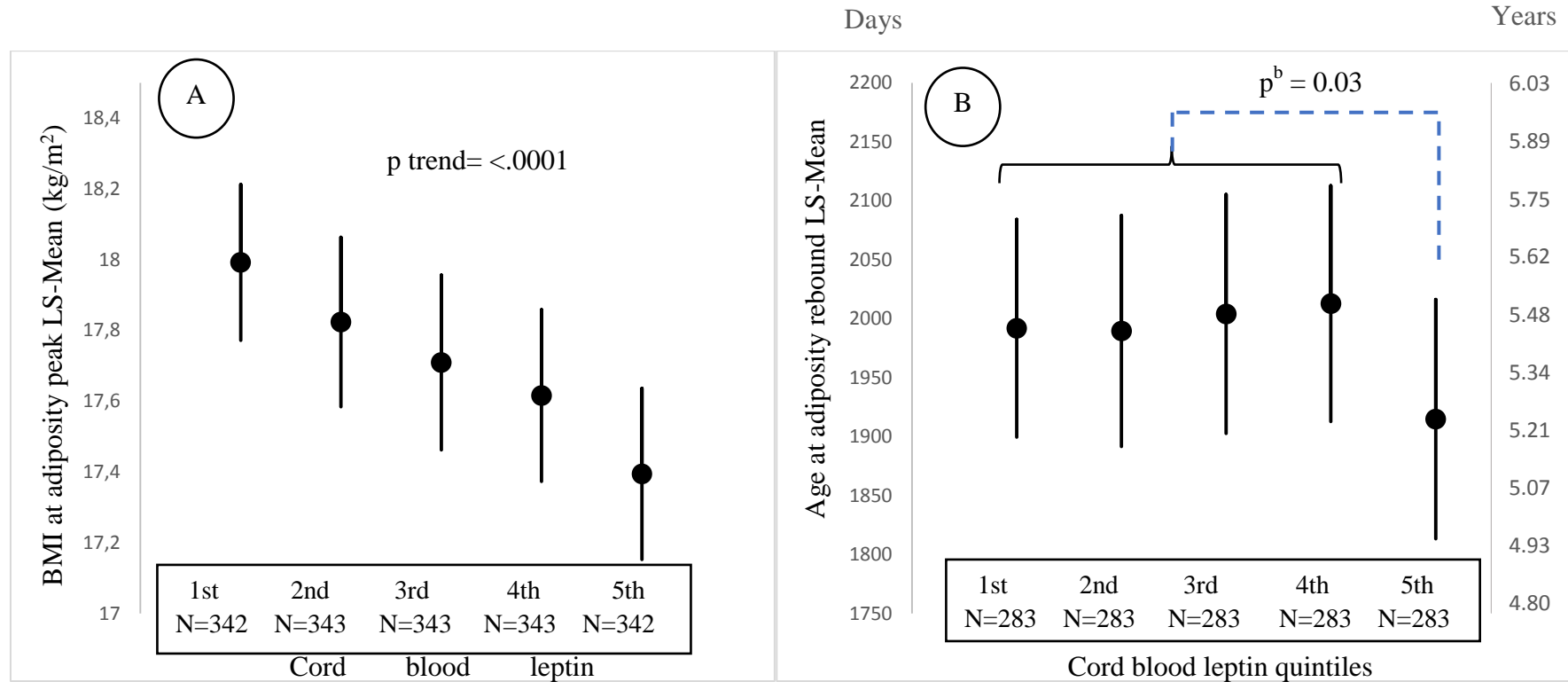


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**Figure 1: Flow chart of the children included in the study.** Two separate samples based on the outcomes with adiposity peak on the left and adiposity rebound on the right. BMI, body mass index; SNP, single nucleotide polymorphism



**Figure 2: Adjusted means<sup>a</sup> (SE) for A) BMI at adiposity peak (AP) and B) age at adiposity rebound (AR) according to cord leptin level quintiles<sup>b</sup>.**

<sup>a</sup> 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.

<sup>b</sup> Quintiles 1, 2, 3 and 4 were grouped together and compared to the last quintile in the model

**Table 1: Description of study populations**

	<b>Children with estimated BMI at AP N=1713</b>	<b>Children with estimated age at AR N=1415</b>
	Mean (SD) or % (N)	Mean (SD) or % (N)
<b>Sex (boys)</b>	51.8 (888)	52.3 (746)
<b>Preterm birth (yes)</b>	5.4 (92)	5.5 (78)
<b>Birth weight (kg)</b>	3.3 (5.1)	3.3 (5.0)
<b>Birth size groups <sup>a</sup></b>		
<b>SGA</b>	13.1 (218)	13.3 (184)
<b>AGA</b>	78.4 (1309)	77.4 (1070)
<b>LGA</b>	8.6 (143)	9.3 (128)
<b>Gestational weight gain (kg)</b>	13.4 (4.7)	13.3 (4.7)
<b>Gestational diabetes (yes)</b>	6.65 (94)	6.60 (113)
<b>Maternal BMI (kg/m<sup>2</sup>)</b>	23.2 (4.6)	23.1 (4.4)
<b>Paternal BMI (kg/m<sup>2</sup>)</b>	25.1 (3.7)	25.1 (3.6)
<b>Never smoking during pregnancy</b>	75.8 (1269)	78.6 (1088)
<b>Maternal age at delivery (years)</b>	29.7 (4.8)	29.9 (4.7)
<b>Maternal educational level (years)</b>	13.7 (2.6)	14.0 (2.6)
<b>Paternal educational level (years)</b>	13.1 (2.6)	13.3 (2.6)
<b>Age at AR (years)</b>	5.5 (1.4)	5.5 (1.4)
<b>BMI at AP (kg/m<sup>2</sup>)</b>	17.5 (1.3)	17.5 (1.3)
<b>Cord blood leptin level (ng/ml)</b>	11.1 (11.0)	11.0 (10.5)
<b>LEPR SNP rs9436303</b>		
<b>A/A</b>	55.4 (668)	55.0 (551)
<b>A/G</b>	36.9 (445)	36.8 (369)
<b>G/G</b>	7.6 (92)	8.2 (82)
<b>G-allele frequency</b>	26.1	26.6

<sup>a</sup> 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/m<sup>2</sup>, N=1713<sup>a</sup>) and age at AR (in days, N=1415<sup>b</sup>)**

Characteristics	Separate and unadjusted models <sup>c</sup>		Separate adjusted model with leptin level <sup>d</sup>		Separate adjusted model with <i>LEPR</i> SNP <sup>d</sup>		Fully adjusted model <sup>d</sup>	
	$\beta$ (SE)	p value	$\beta$ (SE)	p value	$\beta$ (SE)	p value	B (SE)	p value
<b>Outcome = BMI at AP; N=1713<sup>a</sup></b>								
Birth size groups <sup>e</sup> (ref=AGA)								
SGA			-0.57 (0.10)	<.0001	-0.48 (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
Cord blood leptin (log-transformed)	-0.14 (0.04)	0.0001	-0.19 (0.04)	<.0001			-0.19 (0.04)	<.0001
<i>LEPR</i> SNP rs9436303 (number of G alleles carried)	0.25 (0.06)	<.0001			0.24 (0.06)	<.0001	0.24 (0.06)	<.0001
<b>Outcome = Age at AR; N=1415<sup>b</sup></b>								
Birth size groups <sup>e</sup> (ref=AGA)								
SGA			-101.0 (39.8)	0.01	-94.0 (39.0)	0.02	-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
Cord blood leptin (log-transformed)	-34.9 (15.0)	0.02	-24.7 (17.6)	0.16			-22.7 (17.6)	0.20
<i>LEPR</i> 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

<sup>a</sup> Sample of children with estimated BMI at AP, linear regression model after multiple imputation on missing data of leptin, *LEPR* SNP and covariates.

<sup>b</sup> Sample of children with estimated age at AR, linear regression model after multiple imputation on missing data of leptin, *LEPR* SNP and covariates.

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

<sup>d</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>e</sup> 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  $i^{\text{th}}$  individual at the  $t^{\text{th}}$  time point, whatever the period considered (12).

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

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,
- $\varepsilon_{it}$  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 <sup>a</sup>	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 Jeness-Bayley modelling <sup>b</sup>	Continuous		0
Height predicted at 5 years by Jeness-Bayley modelling <sup>b</sup>	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

<sup>a</sup> Fully conditional specification method.

<sup>b</sup> Predicted weight and height were calculated by using previously modelled trajectories from the Jeness–Bayley model (1, 2).

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**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	P >  t  or $\chi^2$	Included N=1415	Excluded N=587	P >  t  or $\chi^2$
	Mean (SD) or % (N)			Mean (SD) or % (N)		
Sex (boys)	51.8 (888)	59.0 (112)	0.06	52.7 (746)	52.1	0.80
Preterm birth (yes)	5.4 (92)	9.4 (18)	0.02	5.5 (78)	6.5 (32)	0.41
Birth weight (kg)	3.3 (0.5)	3.2 (0.5)	0.13	3.3 (0.5)	3.2 (0.5)	0.03
Birth size groups <sup>a</sup>			0.38			0.04
SGA	13.1 (218)	15.3 (28)		13.3 (184)	13.2 (62)	
AGA	78.4 (1309)	78.7 (144)		77.4 (1070)	81.3 (383)	
LGA	8.6 (143)	6.0 (11)		9.3 (128)	5.5 (26)	
Gestational weight gain (kg)	13.4 (4.7)	13.7 (5.7)	0.41	13.3 (4.7)	13.8 (5.3)	0.05
Gestational diabetes (yes)	6.65 (94)	5.92 (29)	0.57	6.60 (113)	5.21 (10)	0.46
Maternal BMI (kg/m <sup>2</sup> )	23.2 (4.6)	23.5 (5.2)	0.43	23.1 (4.4)	23.6 (5.1)	0.10
Paternal BMI (kg/m <sup>2</sup> )	25.1 (3.7)	24.4 (3.4)	0.02	25.1 (3.6)	24.7 (3.7)	0.03
Never smoking during pregnancy	75.8 (1269)	54.0 (94)	<.0001	78.6 (1088)	59.4 (275)	<.0001
Maternal age at delivery (years)	29.7 (4.8)	27.9 (5.4)	<.0001	29.9 (4.7)	28.2 (5.2)	<.0001
Maternal educational level (years)	13.7 (2.6)	12.3 (2.5)	<.0001	14.0 (2.6)	12.5 (2.5)	<.0001
Paternal educational level (years)	13.1 (2.6)	12.2 (2.2)	<.0001	13.3 (2.6)	12.2 (2.4)	<.0001

Age at AR (years)				5.5 (1.4)			Comparison between children with estimated BMI at AP and
BMI at AP (kg/m <sup>2</sup> )	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	
<i>LEPR</i> SNP rs9436303			0.53			0.80	
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)		

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

<sup>a</sup> 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

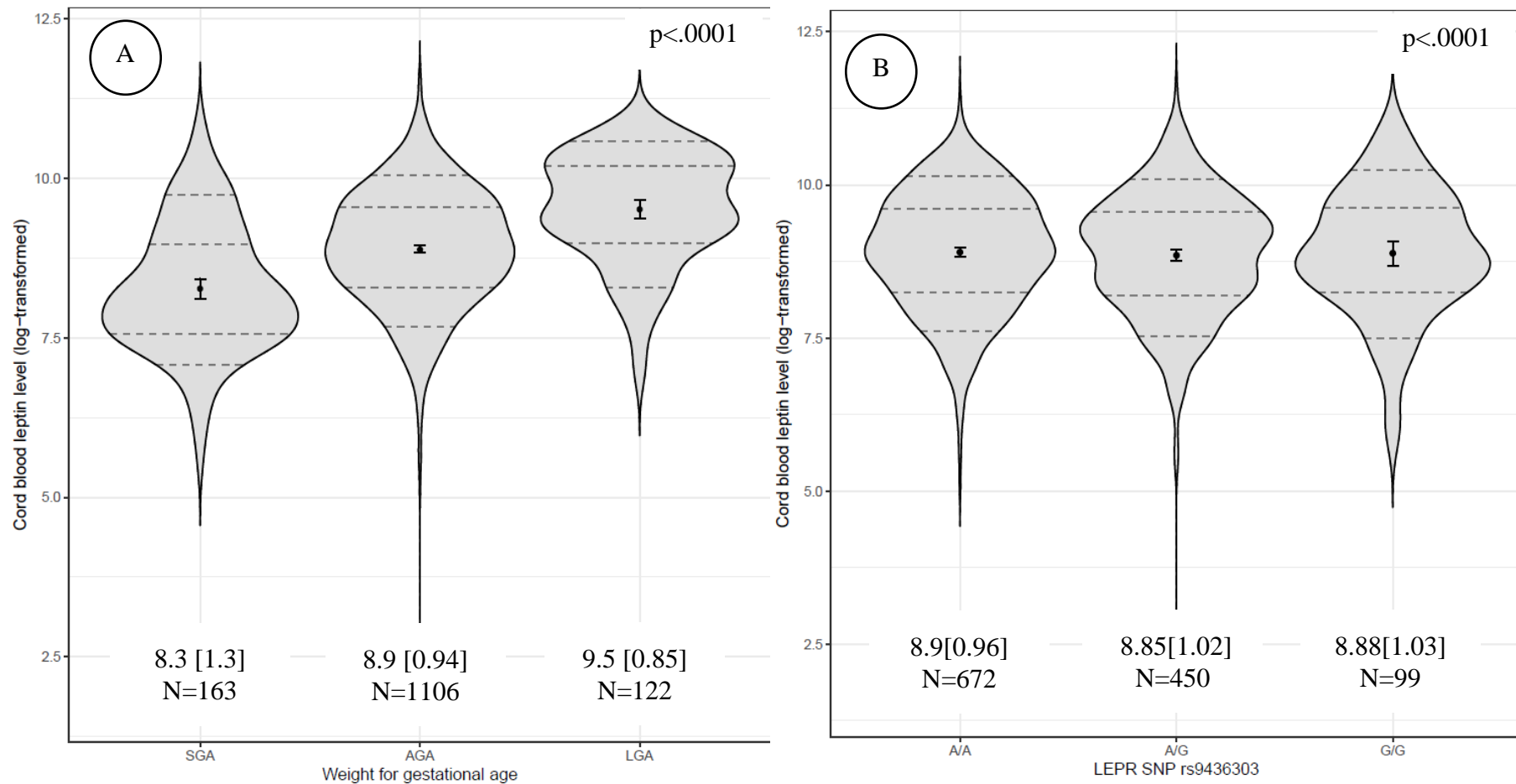
**Supplementary Table S3: Results of association analyses of complete cases between cord blood leptin level, *LEPR* SNP rs9436303 SNP and BMI at AP and age at AR**

Characteristics	Separate and unadjusted models <sup>a</sup>		Separate adjusted model with leptin level <sup>b</sup>		Separate adjusted model with <i>LEPR</i> SNP <sup>b</sup>		Fully adjusted model <sup>b</sup>	
	$\beta$ (SE)	p value	$\beta$ (SE)	p value	$\beta$ (SE)	p value	B (SE)	p value
<b>Outcome = BMI at AP</b>			N=1124		N=1047		N=948	
Birth size groups <sup>c</sup> (ref=AGA)								
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
<i>LEPR</i> SNP rs9436303 (number of G alleles carried)	0.24 (0.06)	<.0001			0.22 (0.07)	0.001	0.24 (0.07)	0.001
<b>Outcome = Age at AR</b>			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
<i>LEPR</i> 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

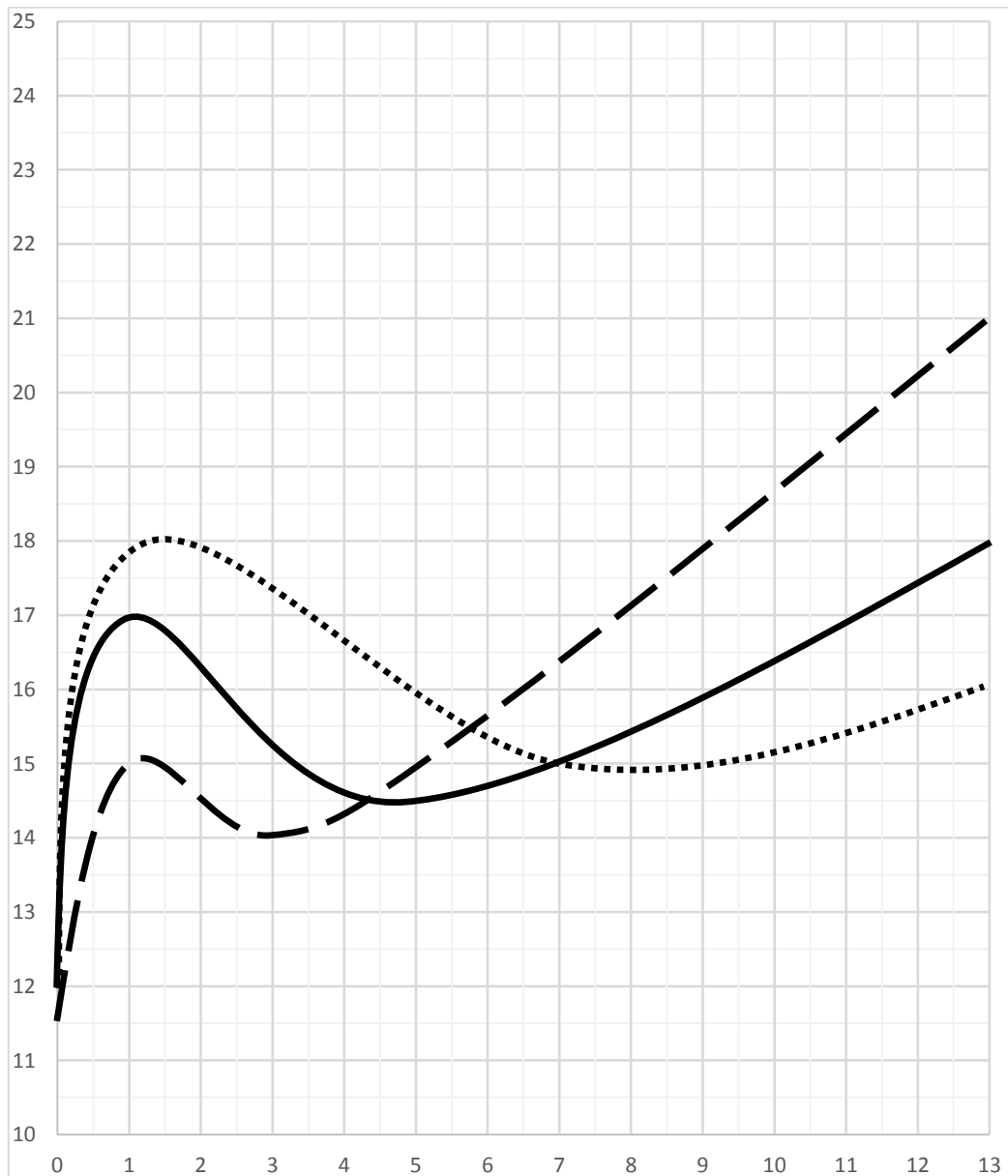
<sup>a</sup> Two separate models for cord blood leptin and rs9436303 without any adjustment.

<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.*