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Early-life risk factors and their cumulative effects as predictors of overweight in Spanish children.

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Abstract:	Objectives: To explore early-life risk factors of overweight/obesity at age 6y and their cumulative effects on overweight/obesity at ages 2-,4- and 6y. Methods: 1,031 Spanish children were evaluated at birth and during a 6-year follow-up. Early-life risk factors included: parental overweight/obesity, parental origin/ethnicity, maternal smoking during pregnancy, gestational weight gain, gestational age, birth weight, caesarean section, breastfeeding practices and rapid infant weight gain collected via hospital records. Cumulative effects were assessed by adding up those early-risk factors that significantly increased the risk of overweight/obesity. We conducted binary logistic regression models. Results: Rapid infant weight gain (OR=2.29, 99%CI=1.54-3.42), maternal overweight/obesity (OR=1.93, 99%CI=1.27-2.92), paternal overweight/obesity (OR=2.17, 99%CI=1.44-3.28), Latin American/Roma origin (OR=3.20, 99%CI=1.60-6.39) and smoking during pregnancy (OR=1.61, 99%CI=1.01-2.59) remained significant after adjusting for confounders. A higher number of early-life risk factors accumulated was associated with overweight/obesity at age 6y but not at age 2y and 4y. Conclusions: Rapid infant weight gain, parental overweight/obesity, maternal smoking and origin/ethnicity predict childhood overweight/obesity and present cumulative effects. Monitoring children with rapid weight gain and supporting a healthy parental

	weight are important for childhood obesity prevention.

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Abstract

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Conclusions: Rapid infant weight gain, parental overweight/obesity, maternal smoking and origin/ethnicity predict childhood overweight/obesity and present cumulative effects. Monitoring children with rapid weight gain and supporting a healthy parental weight are important for childhood obesity prevention.

Keywords: overweight; children; early-life risk factors; prevention, pregnancy.

Abbreviations: SES, Socio-Economic Status; BMI, Body Mass Index; CALINA, Growth and Feeding during Infancy and Early Childhood in Children from Aragon; OR, Odds Ratio; SD, Standard Deviation; WHO, World Health Organization.

Early-life risk factors and their cumulative effects as predictors of overweight in Spanish children

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Introduction

Childhood obesity is a major public health problem (Gupta et al. 2012). Despite reported stabilization of its prevalence in developed countries, overall trends in childhood obesity mask significant, increasing differences between children from upper and lower socioeconomic status (SES) backgrounds and in those who accumulate more risk factors (White et al. 2016). Pre-, peri and postnatal risk factors have been revealed as determinants of subsequent childhood overweight/obesity (Reilly et al. 2005). Hence, infancy constitutes a critical period for future preventive strategies mainly in most deprived groups (Parrino et al. 2016). Several early-life risk factors have been identified in the literature, including maternal pre-pregnancy overweight/obesity, parental origin/ethnicity, smoking during pregnancy, excess gestational weight gain, prematurity, high birth weight, caesarean section, not being breastfed and rapid infant weight gain (Bammann et al. 2014; Iguacel et al. 2017). While a meta-analysis found significant and strong independent associations with childhood overweight for maternal pre-pregnancy overweight, smoking during pregnancy and high infant birth weight, there was inconclusive evidence for caesarean section and breastfeeding practices (Lefebvre and John 2014; Weng et al. 2012; Yan et al. 2014).

These risk factors tend to cluster in socially patterned ways and may confound results. For example, mothers with a low educational level are more likely to smoke during pregnancy, which increases the risk of preterm birth, and thereby reduces the probability of breastfeeding (Heck et al. 2006; Mangrio et al. 2011; Oves Suarez et al. 2014). Several studies investigated the influence of early-life risk factors on childhood obesity; however, most of them did not adjust for potential confounding factors and therefore, did not discriminate their real contribution to childhood obesity (Stettler et al. 2000). Children presenting overweight/obesity tend to cumulate several risk factors, consequently it is important to evaluate their possible combined effects in order to design public policies tackling major modifiable risk factors (Robinson et al. 2015).

Moreover, most studies have focused on one or few early-life risk factors associated with obesity assessed at one particular age (Barros et al. 2012; Dennison et al. 2006). To the best of our knowledge this is the first paper examining most important identified early-life risk factors in the literature in a cohort of Spanish children followed from birth to age 6 and evaluated at 3-time points (at age 2, 4 and 6 years). Moreover, we have examined the combined effect that most significant early-life risk factors identified in this study have on the risk of developing overweight/obesity.

Therefore, this study aimed to explore (i) the impact of early-life risk factors on the subsequent risk of obesity at 6 years old in a cohort of Spanish children participating in the Growth and Feeding during Infancy and Early Childhood in Aragon (CALINA) study, and (ii) the association between the number of early-life risk factors and presenting overweight/obesity at age 2, 4 and 6.

Methods

Design and study population

CALINA is an ongoing birth cohort study whose sampling design is described elsewhere in detail (Oves Suarez et al. 2014). CALINA´s study main objective was to assess growth patterns, body composition and feeding aspects in infants and children and to examine prenatal, postnatal and socio-cultural factors which may influence them. The cohort was randomly drawn from births occurring from March 2009 to February 2010 in different localities in the region of Aragon (Spain), recruited from Primary Care Centres by trained pediatric staff and with compliance and attendance over 80% of the population living in this area. The study sample is a representative cohort of the Aragonese population, which presents similar childhood obesity rates to other northern regions in Spain (Serra-Majem et al. 2006). 1,630 families were contacted to participate in the CALINA study. 1,602 accepted to participate; of which, 1,540 new-born infants without any malformation, diseases or physical disabilities who had information on sex, birth weight, length at birth, and date and place of birth were examined at birth and periodically re-examined at 2 weeks, monthly and yearly. After 6-year follow-up 323 children did no longer participate in our study (retention rate 79%). Children with missing values in exposures, covariates or outcomes at baseline or follow-up were excluded. Asians were not included because models could not run satisfactorily due to the small size of the sample that led to unstable results. Finally, the analysis included 1,031 children (54.2% boys; **Figure 1**). An analysis was conducted on participants who were not included in the analysis and results confirmed children who had a migrant background and lower parental education were more likely to not participate in follow-up examinations.

Parents or legal guardians gave written informed consent for examinations for their children. Ethical approval was obtained from the regional Committee of Ethics (Comité Ético de Investigación Clínica de Aragón, CEICA).

Measurements

Outcome Measure

Height and weight were obtained by trained staff using the same SECA® device at different time points (at birth, at 2 weeks, monthly -at 1, 2, 4, 6 and 9 months- and yearly -at 1, 2, 4 and 6 years-). Barefoot body height was measured in cm to the nearest 0.1 cm and body weight in kg to the nearest 10 g, with children in a fasting state and wearing light clothes. To calculate age- and sex-specific BMI z-scores from birth to 5 years we used child growth standards tables of WHO, using >+2 standard deviation (SD) and > +3 SD for overweight and obese (World Health Organization 2007) and to calculate age- and sex-specific BMI z-scores at age 6 we used WHO growth reference tables established for children aged 5 to 19 years using > +1SD, > +2SD for overweight and obese (World Health Organization. 2007).

Early risk factors and sociodemographic characteristics

Early-life risk factors were divided into prepartum, peripartum and postpartum factors:

Prepartum factors:

Maternal and paternal body mass index (BMI) and parental origin/ethnicity were obtained by a face-to-face interview with parents. Mother's tobacco use during pregnancy and gestational weight gain were obtained from medical records.

(i) *Maternal body mass index (BMI)* and (ii) *paternal body mass index (BMI):* Parents reported their pre-pregnancy weight and height and we calculated maternal and paternal BMI as weight (kg) divided by height squared (m^2) and classified as normal weight, <25 kg/m^2; overweight, 25-<30 kg/m^2; and obese, ≥30 kg/m^2.

(iii) Parental origin/ethnicity: Mothers also reported their ethnicity/origin and children were classified as Spanish Roma/gypsies, Eastern Europeans, Latin Americans (Central, South America), Africans (North Africa, Sub-Saharan Africa) and non-Roma Spanish children. In 94% of children, the category of both parents was the same and, then, was

used as the origin/ethnicity. In those cases in which the minority group status of the 2 parents differed, it was based on mothers´ origin/ethnicity.

(iv) *Maternal smoking status during pregnancy*: mother was considered as smoker if she smoked over pregnancy, regardless of the number of cigarettes. Physicians obtained the data by interviewing mothers before hospital discharge after delivery and by abstracting medical records.

(v) *Gestational weight gain* was obtained from medical records, which was calculated as the difference between maximum recorded weight during pregnancy and self-reported pre-pregnancy weight (determined at first antenatal visit). Thereafter, gestational weight gain was categorized as excessive, adequate and insufficient based on the 2009 Institute of Medicine (IOM) recommendations for healthy weight gain for pregnant women, by pre-pregnancy BMI category: 12.5-18.0 kg for women with a BMI <18.5; 11.5-16.0 kg for women with a BMI 18.5-24.9; 7.0-11.5 kg for women with a BMI 25.0-29.9 and 5.0-9.0 kg for women with a BMI <30.0 (Institute of Medicine and National Research Council Committee 2009).

Peripartum factors gathered from hospital records:

(i) *Gestational age* was categorized into <37 weeks (preterm) and 37-42 weeks (term).

(ii) *Birth weight*, categorized as low (<2.5 kg), normal (2.5-<4 kg) and high (≥4 kg) (Zhang et al. 2016).

(iii) *Delivery mode*, categorized as caesarean section or not.

Postpartum factors obtained from medical records:

(i) Early rapid infant body weight gain. Age- and sex-specific weight z-scores at birth and at 6 months of age were calculated using WHO (World Health Organization 2006) child growth standards tables. We assessed infant gain weight as gain in weight z-score between birth and six months of life. Early rapid infant body weight gain was considered as an increase in body weight z-score above +0.67 SD from birth to 6 months of age (Ong and Loos 2006).Early rapid infant weight gain was considered as an increase in body weight z-score above +0.67 SD from birth to 6 months of age (Ong and Loos 2006).

(ii) Exclusive breastfeeding for at least four months was defined as giving breastmilk as the only infant food source with no other liquids or food given according to WHO (World Health Organization 2010).

Sociodemographic characteristics included sex and age of children and education attained by parents. Mothers and fathers reported their highest level of education. Categories were coded according to International Standard Classification of Education (ISCED-1997) and re-categorized into: low (0-2), medium (3-4) and high (5-6) ISCED educational levels (UNESCO Statistics 1997).

Statistical Analyses

Sociodemographic information was compared using chi-square statistics for categorical variables. To study the impact of early-life risk factors on the risk of children´s excess of weight we carried out a multivariable analysis in two stages. Firstly, we built binary logistic regression models for each early risk factor included in the present study to assess the associations with childhood excess body weight at age 6. Basic model adjustment included sex and age at measurement, and full adjustment model included the basic model plus the possible confounders for each early-life risk factor that have been found to be relevant in the literature (i.e. maternal BMI was adjusted for maternal education, ethnicity/origin, and maternal smoking during pregnancy. The complete list of confounding factors is displayed in Table 2. Secondly, to assess the combined effect of all statistically significant early-life risk factors binary logistic regression models were run. As these risk factors could be correlated, only early-life risk factors that were statistically significant at 6 years old ($p<0.01$) in previous first step analyses were included in the following models (maternal BMI $\geq$25 kg/m^2 and paternal BMI$\geq$25 kg/m^2, Latin American or Roma origin, maternal smoking, not being exclusively breastfeed during the first 4 months and rapid infant weight gain). These models were adjusted for sex, age and parental education and each factor included in the model. Additionally, three longitudinal analyses were conducted to assess the association between the accumulation of these early risk factors seemingly influential in the analysis at 6 years old and childhood excess body weight at 2, 4 and 6 years old. In this case, binary logistic regressions were run adjusting for sex and age at measurement, maternal and paternal education. To test the effect of the accumulation of early-life risk factors on childhood overweight and obesity we added up those early-risk factors significantly increasing the risk of being overweight/obese at 6 years old: Latin American or Roma origin, maternal BMI ≥25 kg/m^2, paternal BMI ≥25 kg/m^2, maternal smoking during pregnancy, not being exclusively breastfeed during the first 4 months and early rapid infant weight gain. The total number of early-life risk factors ranged from 0 (no risk factors) to 6 (all six risk factors) and was divided into five categories (four to six risk factors, three risk factors, two risk factors, one risk factor and no risk factors).

Furthermore, before model building, correlations among early-life risk factors were checked ranging from 0.12 (between birthweight and caesarean section) to 0.34 (between gestational age and birthweight). The reference category used was underweight/normal weight-for each outcome (overweight and obesity risk).

The significance level was set at 0.01 to account at least partially for multiple testing. Analyses were performed using Statistical Package for the Social Sciences (version 22.0; SPSS, Inc.).

Results

Table 1 summarizes descriptive characteristics of children and parents according to weight status (normal weight vs. overweight/obesity) of children at 6-year follow-up. The percentage of children presenting normal weight (including also children underweight) at age 6 was 68.4% (28.9% of total number of children were underweight, data not shown).

Table 2 presents OR and 99%CI for the associations between early-life risk factors and excess body weight in children at 6 years old for basic and fully adjusted models. In the fully adjustment models, children whose mothers were overweight (OR 1.91, 99%CI 1.38-2.66) or obese (OR 2.20, 99%CI 1.41-3.42) were more likely to be overweight at age 6 than children whose mothers were normal weight/underweight. Similarly, children whose fathers were overweight (OR 2.10, 99%CI 1.59-3.00) or obese (OR 3.05, 99%CI 2.00-4.64) were more likely to be overweight at age 6 than children whose fathers were normal weight/underweight. Roma children (OR 4.87, 99%CI 2.00-11.81) and children with Latin American background (OR 3.22, 99%CI 1.79-5.77) were more likely to be overweight or obese at age 6 compared with non-Roma Spanish children regardless of confounders. Children whose mothers reported to have smoked during pregnancy were more likely to be overweight/obese at age 6 than children whose mothers did not smoke during pregnancy (OR 1.59, 99%CI 1.03-2.43). Children who experienced rapid weight gain from birth to 6 months of age were more likely to be overweight/obese at 6 years old than children who did not experience rapid infant weight gain (OR 3.39, 99%CI 2.03, 5.65). In the basic model, exclusive breastfeeding for 4 months was found to be associated with lower risk of being overweight/obese at 6 years old (OR 1.34, 99%CI 1.01-1.82). However, when adjusting for parental BMI, maternal education, maternal smoking during pregnancy and parental origin/ethnicity, this risk was reduced and it was no longer significant (OR 1.20, 99%CI 0.82-1.75).

Table 3 shows the combined effect of all factors found statistically significant in previous fully adjusted models regarding children´s excess of weight at 6 years old. The multivariable model included maternal BMI ≥25 kg/m^2, paternal BMI≥25 kg/m^2, Latin American or Roma origin, maternal smoking, not being exclusively breastfeed during the first 4 months and rapid infant weight gain. After adjusting for sex, age, maternal and paternal education and every early-life risk factor, maternal BMI>25 kg/m^2 (OR 1.93, 99%CI 1.27-2.78), paternal BMI>25 kg/m^2 (OR 2.08, 99%CI 1.06-2.51), Latin American origin/Spanish Roma (OR 3.20, 99%CI 1.60-6.39) and early rapid infant weight gain (OR 2.09,99%CI 1.54, 3.42) remained as significant predictors of overweight/obesity at age 6.

Table 4 shows OR and 99%CI for the associations between the number of early-life risk factors found statistically significant with overweight/obesity at age 6 based on previous analyses (maternal BMI≥25, paternal BMI≥25, Latin American or Roma origin, smoking during pregnancy, non-exclusive breastfeeding during the first 4 months and early postnatal rapid infant weight gain) and excess body weight in children at 2, 4 and 6 years old. A higher number of early-life risk factors was associated with higher odds of being overweight or obese at age 6, where OR increased with the number of early-life risk factors: two early-life risk factors (OR 2.72, 99%CI 1.54-3.42); three early-life risk factors (OR 5.02, 99%CI 2.28-11.04) and four to six early-life risk factors (OR 7.33, 99%CI 3.01-17.84). No significant associations were found at age 2 or 4 years.

Discussion

This study investigated both the impact of early-life risk factors on later overweight and obesity in Spanish children at 6 years old and their cumulative effect on the risk of becoming overweight/obese at 2, 4 and 6 years old.

Maternal BMI, paternal BMI, parental origin/ethnicity, maternal smoking during pregnancy and rapid infant weight gain were statistically significant independent factors of childhood overweigh and obesity in our investigation after adjusting for confounding factors and these early-life risk factors had an accumulative effect on overweight and obesity in children who were aged 6 years old. Particularly, there was a 7-fold increase in the risk of being overweight or obese at age 6 for children who had 4 or more risk factors, compared with children who had none.

Parental BMI and parental origin/ethnicity also confounded many of the associations studied and were strong risk factors for childhood obesity, as other investigations have revealed (Parikka et al. 2015). Parental overweight and obesity could influence the risk of obesity in their descendants due to shared genes and environmental factors within families (Whitaker et al. 1997; Williams et al. 2017). The association between parental origin/ethnicity and childhood obesity could be due genetic and cultural diversity in minority groups and those related to SES could result in differences in Energy balance-related behaviours. These groups are at higher risk of adopting an unhealthy diet, insufficient physical activity and sedentary behaviours, explaining differences found in overweight and obesity prevalence among these groups. Minorities groups (particularly, Roma/gypsies and Latin Americans) are more exposed to more vulnerabilities possibly leading to inadequate adaptation in obesogenic environments characterized by low levels of physical activity, high energy density diets and a sedentary lifestyle compared with non-vulnerable groups (Iguacel et al. 2017). Maternal educational level was used as an indicator of socioeconomic status because several studies found maternal educational level to be a reliable determinant of children's dietary behaviour and childhood obesity (van Ansem et al. 2014).

Along with our results, consistent evidence has been shown in previous studies regarding smoking during pregnancy as a risk factor for childhood overweight/obesity (Oken et al. 2008). Via intrauterine, exposure to smoke results in prenatal undernutrition. This nutritional deprivation may lead to increased nutrient achievement later and finally postnatal obesity (Oken et al. 2008). The pooled estimate from unadjusted odds ratios was higher to the adjusted estimate, suggesting maternal education and parental origin/ethnicity between smokers and non-smokers explained just partly the association.

Previous meta-analyses have showed that due to permanent alterations in metabolism excessive gestational weight gain is significantly associated with childhood overweight/obesity (Mamun et al. 2014; Tie et al. 2014). However, we did not find statically significant associations between an excessive gestational weight gain and offspring overweight, even though associations pointed to the expected directions (Sridhar et al. 2014). Part of the risk of an excessive gestational weight of childhood overweigh has been related to maternal pre-pregnancy BMI (Samura et al. 2016). Our models were adjusted for maternal pre-pregnancy BMI, which may be the result of this lack of significance as other studies have stated (Samura et al. 2016).

Concerning gestational age, we did not find any statistically significant association between gestational age and childhood obesity. In the literature, there is mixed evidence on whether gestational age is linked or not with childhood overweight/obesity (Heppe et al. 2013). Arguably infants born preterm usually compensate by engaging in rapid infant weight gain in early-life and this ‘catch-up’ growth is associated with an increased risk of childhood obesity. Attending previous investigations therefore, this association could be explained mainly due to early postnatal rapid infant weight gain and not directly because of gestational age (Cho and Suh 2016) but such result in not shown in our analysis.

Low and high birth weight have been associated with subsequent childhood obesity through increased leptin levels after catch-up growth during childhood and programming for lean mass respectively (Danielzik et al. 2004; Jornayvaz et al. 2016). Maternal glucose levels during pregnancy could also explain the association between birthweight and offspring of obesity. In fact, an excess of fetal insulin, due to maternal hyperglycaemia, might work as a growth hormone for the fetus and can also alter the expression of hypothalamic neurotransmitter leading to an increase in the appetite and later obesity (Guillmann 2003). However, we did not find statically significant associations between high birth weight and future overweight in children. In our analysis, only one child weighted five kg and most children who were categorized as high birthweight weighted around four kg, which could partially explain the lack of significance. Despite this lack of significance, results pointed to the expected directions and higher ORs in children who weighted more than 4 kg at birth were observed.

Birth by caesarean section has been implicated in the development of childhood obesity (Yuan et al. 2016). A recent meta-analysis has reported children born by caesarean section are at higher risk of developing obesity in childhood and this association remained significant after accounting for major confounding factors (Kuhle et al. 2015). Despite these findings, we could not find any statistically significant association between birth by caesarean section and childhood obesity.

Breastfeeding has yielded inconsistent results in the literature. Some studies have reported breastfed children have lower risk of childhood obesity than those who have not been breastfed (Yan et al. 2014) while others have stated that evidence from these studies could be influenced by confounding factors and therefore breastfeeding would not be likely to be a protective factor for childhood obesity (Lefebvre and John 2014). Our study found a raw effect between not being exclusively breastfeed in the first 4 months and future childhood overweight. Nevertheless, this effect disappeared when adjusting for maternal BMI, maternal smoking during pregnancy and education, suggesting protective effect of breastfeeding against childhood obesity could be due to confounding variables.

Early rapid infant weight gain has been reported to be a risk factor of childhood obesity (Ong and Loos 2006), which is in line with the results of our study. This factor had an independent effect on obesity risk at 6y and remained statically significant after adjusting for confounding factors and exclusive breastfeeding for at least 4 months. Specifically, rapid infant weight gain from birth to 6 months of age was the strongest predictor of later risk of childhood overweight/obesity in our study.

Finally, we examined the cumulative effect of early-life risk factors found statically significant in our study (maternal smoking during pregnancy, non-exclusive breast feeding during the first 4 months, rapid infant weight gain from birth to 6 months of age, maternal BMI >25, paternal BMI >25 and Latin American/Roma origin) in childhood overweight and obesity. Children who accumulated more risk factors had higher risk of being overweight/obese at age 6. Particularly, there was a 7-fold increase in the risk of overweight in children with 4 to 6 risk factors, 5-fold in children with 3 risk factors and 2-fold in children with 2 risk factors compared with children who had no risk factors and after adjusting for sex, age and maternal education. However, these effects were not observed at 2 and 4 years old, suggesting this tendency seems to become more pronounced over time, which have been suggested in other studies.(Robinson et al. 2015; Salsberry and Reagan 2005). These results are likely due to the combination in a same subject of both expression of genetic predisposition and being more time exposed to obesogenic environments. Furthermore, we hypothesized that children who tend to accumulate more early-life risk factors are probably more subject to socioeconomic vulnerabilities for a longer period, which may explain increased prevalence of overweight/obesity in these groups over time. Health behaviours related to obesity such as physical activity, diet or sleep and mental health can worsen due to stressful events and household dysfunction that might characterize ethnic minority and low SES groups (Iguacel et al. 2017).

Some limitations of this study should be acknowledged. Firstly, the CALINA study is not representative of Spanish population since Aragon covered a limited geographic area within the country and results might not be extrapolated to the whole population. Another limitation is reliance on self-report measures for parents (parental weight-height and education). Moreover, a selection bias cannot be precluded as there were participants (mainly children whose parents were originally from Eastern European countries, Africa and Latin America and had lower parental education) who did not complete all information required or did not continue the study at follow-up. Furthermore, some very important confounding factors such as dietary intake, income and parity were not reported and thus results must be interpreted with caution. Finally, some associations were not found to be statically significant maybe due to the small size of some groups studied (i.e. in children who weighted more than 4 kg at birth). A special strength of the study is that to our knowledge, this is the first paper investigating early-life risk factors and their accumulative effect at 2, 4 and 6 years old using a Spanish cohort in a 6 years follow-up. The prospective collection of data on a wide range of risk factors extending from pregnancy through infancy and the ability to adjust for confounding factors are also strengths of this study.

Conclusion

Parental origin/ethnicity, parental overweight and obesity, smoking during pregnancy and rapid infant weight gain were important determinants of childhood overweight/obesity. All these risk factors have cumulative effects and tend to cluster in socially patterned ways. However, these effects were not observed when children were 2 and 4 years, suggesting this tendency become more pronounced over time as children are more time exposed to these risk factors and the obesogenic environments. Therefore, the first year is critical for childhood obesity development, and its prevention. Strategies such as monitoring children with rapid infant weight gain, supporting attainment of a healthy parental weight and preventing smoking during pregnancy could be of importance for preventing childhood obesity.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Research involving human participants Ethical approval was obtained from the regional Committee of Ethics (Comité Ético de Investigación Clínica de Aragón, CEICA).

Informed consent Parents or legal guardians gave written informed consent for examinations for their children.

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Early-life risk factors and their cumulative effects as predictors of overweight in Spanish children

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Introduction

Childhood obesity is a major public health problem (Gupta et al. 2012). Despite reported stabilization of its prevalence in developed countries, overall trends in childhood obesity mask significant, increasing differences between children from upper and lower socioeconomic status (SES) backgrounds and in those who accumulate more risk factors (White et al. 2016). Pre-, peri and postnatal risk factors have been revealed as determinants of subsequent childhood overweight/obesity (Reilly et al. 2005). Hence, infancy constitutes a critical period for future preventive strategies mainly in most deprived groups (Parrino et al. 2016). Several early-life risk factors have been identified in the literature, including maternal pre-pregnancy overweight/obesity, parental origin/ethnicity, smoking during pregnancy, excess gestational weight gain, prematurity, high birth weight, caesarean section, not being breastfed and rapid infant weight gain (Bammann et al. 2014; Iguacel et al. 2017). While a meta-analysis found significant and strong independent associations with childhood overweight for maternal pre-pregnancy overweight, smoking during pregnancy and high infant birth weight, there was inconclusive evidence for caesarean section and breastfeeding practices (Lefebvre and John 2014; Weng et al. 2012; Yan et al. 2014).

These risk factors tend to cluster in socially patterned ways and may confound results. For example, mothers with a low educational level are more likely to smoke during pregnancy, which increases the risk of preterm birth, and thereby reduces the probability of breastfeeding (Heck et al. 2006; Mangrio et al. 2011; Oves Suarez et al. 2014). Several studies investigated the influence of early-life risk factors on childhood obesity; however, most of them did not adjust for potential confounding factors and therefore, did not discriminate their real contribution to childhood obesity (Stettler et al. 2000). Children presenting overweight/obesity tend to cumulate several risk factors, consequently it is important to evaluate their possible combined effects in order to design public policies tackling major modifiable risk factors (Robinson et al. 2015).

Moreover, most studies have focused on one or few early-life risk factors associated with obesity assessed at one particular age (Barros et al. 2012; Dennison et al. 2006). To the best of our knowledge this is the first paper examining most important identified early-life risk factors in the literature in a cohort of Spanish children followed from birth to age 6 and evaluated at 3-time points (at age 2, 4 and 6 years). Moreover, we have examined the combined effect that most significant early-life risk factors identified in this study have on the risk of developing overweight/obesity.

Therefore, this study aimed to explore (i) the impact of early-life risk factors on the subsequent risk of obesity at 6 years old in a cohort of Spanish children participating in the Growth and Feeding during Infancy and Early Childhood in Aragon (CALINA) study, and (ii) the association between the number of early-life risk factors and presenting overweight/obesity at age 2, 4 and 6.

Methods

Design and study population

CALINA is an ongoing birth cohort study whose sampling design is described elsewhere in detail (Oves Suarez et al. 2014). CALINA´s study main objective was to assess growth patterns, body composition and feeding aspects in infants and children and to examine prenatal, postnatal and socio-cultural factors which may influence them. The cohort was randomly drawn from births occurring from March 2009 to February 2010 in different localities in the region of Aragon (Spain), recruited from Primary Care Centres by trained pediatric staff and with compliance and attendance over 80% of the population living in this area. The study sample is a representative cohort of the Aragonese population, which presents similar childhood obesity rates to other northern regions in Spain (Serra-Majem et al. 2006). 1,630 families were contacted to participate in the CALINA study. 1,602 accepted to participate; of which, 1,540 new-born infants without any malformation, diseases or physical disabilities who had information on sex, birth weight, length at birth, and date and place of birth were examined at birth and periodically re-examined at 2 weeks, monthly and yearly. After 6-year follow-up 323 children did no longer participate in our study (retention rate 79%). Children with missing values in exposures, covariates or outcomes at baseline or follow-up were excluded. Asians were not included because models could not run satisfactorily due to the small size of the sample that led to unstable results. Finally, the analysis included 1,031 children (54.2% boys; **Figure 1**). An analysis was conducted on participants who were not included in the analysis and results confirmed children who had a migrant background and lower parental education were more likely to not participate in follow-up examinations.

Parents or legal guardians gave written informed consent for examinations for their children. Ethical approval was obtained from the regional Committee of Ethics (Comité Ético de Investigación Clínica de Aragón, CEICA).

Measurements

Outcome Measure

Height and weight were obtained by trained staff using the same SECA® device at different time points (at birth, at 2 weeks, monthly -at 1, 2, 4, 6 and 9 months- and yearly -at 1, 2, 4 and 6 years-). Barefoot body height was measured in cm to the nearest 0.1 cm and body weight in kg to the nearest 10 g, with children in a fasting state and wearing light clothes. To calculate age- and sex-specific BMI z-scores from birth to 5 years we used child growth standards tables of WHO, using >+2 standard deviation (SD) and > +3 SD for overweight and obese (World Health Organization 2007) and to calculate age- and sex-specific BMI z-scores at age 6 we used WHO growth reference tables established for children aged 5 to 19 years using > +1SD, > +2SD for overweight and obese (World Health Organization. 2007).

Early risk factors and sociodemographic characteristics

Early-life risk factors were divided into prepartum, peripartum and postpartum factors:

Prepartum factors:

Maternal and paternal body mass index (BMI) and parental origin/ethnicity were obtained by a face-to-face interview with parents. Mother's tobacco use during pregnancy and gestational weight gain were obtained from medical records.

(i) *Maternal body mass index (BMI)* and (ii) *paternal body mass index (BMI):* Parents reported their pre-pregnancy weight and height and we calculated maternal and paternal BMI as weight (kg) divided by height squared (m^2) and classified as normal weight, <25 kg/m^2; overweight, 25-<30 kg/m^2; and obese, ≥30 kg/m^2.

(iii) Parental origin/ethnicity: Mothers also reported their ethnicity/origin and children were classified as Spanish Roma/gypsies, Eastern Europeans, Latin Americans (Central, South America), Africans (North Africa, Sub-Saharan Africa) and non-Roma Spanish children. In 94% of children, the category of both parents was the same and, then, was

used as the origin/ethnicity. In those cases in which the minority group status of the 2 parents differed, it was based on mothers´ origin/ethnicity.

(iv) *Maternal smoking status during pregnancy*: mother was considered as smoker if she smoked over pregnancy, regardless of the number of cigarettes. Physicians obtained the data by interviewing mothers before hospital discharge after delivery and by abstracting medical records.

(v) *Gestational weight gain* was obtained from medical records, which was calculated as the difference between maximum recorded weight during pregnancy and self-reported pre-pregnancy weight (determined at first antenatal visit). Thereafter, gestational weight gain was categorized as excessive, adequate and insufficient based on the 2009 Institute of Medicine (IOM) recommendations for healthy weight gain for pregnant women, by pre-pregnancy BMI category: 12.5-18.0 kg for women with a BMI <18.5; 11.5-16.0 kg for women with a BMI 18.5-24.9; 7.0-11.5 kg for women with a BMI 25.0-29.9 and 5.0-9.0 kg for women with a BMI <30.0 (Institute of Medicine and National Research Council Committee 2009).

Peripartum factors gathered from hospital records:

(i) *Gestational age* was categorized into <37 weeks (preterm) and 37-42 weeks (term).

(ii) *Birth weight*, categorized as low (<2.5 kg), normal (2.5-<4 kg) and high (≥4 kg) (Zhang et al. 2016).

(iii) *Delivery mode*, categorized as caesarean section or not.

Postpartum factors obtained from medical records:

(i) Early rapid infant body weight gain. Age- and sex-specific weight z-scores at birth and at 6 months of age were calculated using WHO (World Health Organization 2006) child growth standards tables. We assessed infant gain weight as gain in weight z-score between birth and six months of life. Early rapid infant body weight gain was considered as an increase in body weight z-score above +0.67 SD from birth to 6 months of age (Ong and Loos 2006).Early rapid infant weight gain was considered as an increase in body weight z-score above +0.67 SD from birth to 6 months of age (Ong and Loos 2006).

(ii) Exclusive breastfeeding for at least four months was defined as giving breastmilk as the only infant food source with no other liquids or food given according to WHO (World Health Organization 2010).

Sociodemographic characteristics included sex and age of children and education attained by parents. Mothers and fathers reported their highest level of education. Categories were coded according to International Standard Classification of Education (ISCED-1997) and re-categorized into: low (0-2), medium (3-4) and high (5-6) ISCED educational levels (UNESCO Statistics 1997).

Statistical Analyses

Sociodemographic information was compared using chi-square statistics for categorical variables. To study the impact of early-life risk factors on the risk of children´s excess of weight we carried out a multivariable analysis in two stages. Firstly, we built binary logistic regression models for each early risk factor included in the present study to assess the associations with childhood excess body weight at age 6. Basic model adjustment included sex and age at measurement, and full adjustment model included the basic model plus the possible confounders for each early-life risk factor that have been found to be relevant in the literature (i.e. maternal BMI was adjusted for maternal education, ethnicity/origin, and maternal smoking during pregnancy. The complete list of confounding factors is displayed in Table 2. Secondly, to assess the combined effect of all statistically significant early-life risk factors binary logistic regression models were run. As these risk factors could be correlated, only early-life risk factors that were statistically significant at 6 years old ($p<0.01$) in previous first step analyses were included in the following models (maternal BMI $\geq$25 kg/m^2 and paternal BMI$\geq$25 kg/m^2, Latin American or Roma origin, maternal smoking, not being exclusively breastfeed during the first 4 months and rapid infant weight gain). These models were adjusted for sex, age and parental education and each factor included in the model. Additionally, three longitudinal analyses were conducted to assess the association between the accumulation of these early risk factors seemingly influential in the analysis at 6 years old and childhood excess body weight at 2, 4 and 6 years old. In this case, binary logistic regressions were run adjusting for sex and age at measurement, maternal and paternal education. To test the effect of the accumulation of early-life risk factors on childhood overweight and obesity we added up those early-risk factors significantly increasing the risk of being overweight/obese at 6 years old: Latin American or Roma origin, maternal BMI ≥25 kg/m^2, paternal BMI ≥25 kg/m^2, maternal smoking during pregnancy, not being exclusively breastfeed during the first 4 months and early rapid infant weight gain. The total number of early-life risk factors ranged from 0 (no risk factors) to 6 (all six risk factors) and was divided into five categories (four to six risk factors, three risk factors, two risk factors, one risk factor and no risk factors).

Furthermore, before model building, correlations among early-life risk factors were checked ranging from 0.12 (between birthweight and caesarean section) to 0.34 (between gestational age and birthweight). The reference category used was underweight/normal weight-for each outcome (overweight and obesity risk).

The significance level was set at 0.01 to account at least partially for multiple testing. Analyses were performed using Statistical Package for the Social Sciences (version 22.0; SPSS, Inc.).

Results

Table 1 summarizes descriptive characteristics of children and parents according to weight status (normal weight vs. overweight/obesity) of children at 6-year follow-up. The percentage of children presenting normal weight (including also children underweight) at age 6 was 68.4% (28.9% of total number of children were underweight, data not shown).

Table 2 presents OR and 99%CI for the associations between early-life risk factors and excess body weight in children at 6 years old for basic and fully adjusted models. In the fully adjustment models, children whose mothers were overweight (OR 1.91, 99%CI 1.38-2.66) or obese (OR 2.20, 99%CI 1.41-3.42) were more likely to be overweight at age 6 than children whose mothers were normal weight/underweight. Similarly, children whose fathers were overweight (OR 2.10, 99%CI 1.59-3.00) or obese (OR 3.05, 99%CI 2.00-4.64) were more likely to be overweight at age 6 than children whose fathers were normal weight/underweight. Roma children (OR 4.87, 99%CI 2.00-11.81) and children with Latin American background (OR 3.22, 99%CI 1.79-5.77) were more likely to be overweight or obese at age 6 compared with non-Roma Spanish children regardless of confounders. Children whose mothers reported to have smoked during pregnancy were more likely to be overweight/obese at age 6 than children whose mothers did not smoke during pregnancy (OR 1.59, 99%CI 1.03-2.43). Children who experienced rapid weight gain from birth to 6 months of age were more likely to be overweight/obese at 6 years old than children who did not experience rapid infant weight gain (OR 3.39, 99%CI 2.03, 5.65). In the basic model, exclusive breastfeeding for 4 months was found to be associated with lower risk of being overweight/obese at 6 years old (OR 1.34, 99%CI 1.01-1.82). However, when adjusting for parental BMI, maternal education, maternal smoking during pregnancy and parental origin/ethnicity, this risk was reduced and it was no longer significant (OR 1.20, 99%CI 0.82-1.75).

Table 3 shows the combined effect of all factors found statistically significant in previous fully adjusted models regarding children´s excess of weight at 6 years old. The multivariable model included maternal BMI ≥25 kg/m^2, paternal BMI≥25 kg/m^2, Latin American or Roma origin, maternal smoking, not being exclusively breastfeed during the first 4 months and rapid infant weight gain. After adjusting for sex, age, maternal and paternal education and every early-life risk factor, maternal BMI>25 kg/m^2 (OR 1.93, 99%CI 1.27-2.78), paternal BMI>25 kg/m^2 (OR 2.08, 99%CI 1.06-2.51), Latin American origin/Spanish Roma (OR 3.20, 99%CI 1.60-6.39) and early rapid infant weight gain (OR 2.09,99%CI 1.54, 3.42) remained as significant predictors of overweight/obesity at age 6.

Table 4 shows OR and 99%CI for the associations between the number of early-life risk factors found statistically significant with overweight/obesity at age 6 based on previous analyses (maternal BMI≥25, paternal BMI≥25, Latin American or Roma origin, smoking during pregnancy, non-exclusive breastfeeding during the first 4 months and early postnatal rapid infant weight gain) and excess body weight in children at 2, 4 and 6 years old. A higher number of early-life risk factors was associated with higher odds of being overweight or obese at age 6, where OR increased with the number of early-life risk factors: two early-life risk factors (OR 2.72, 99%CI 1.54-3.42); three early-life risk factors (OR 5.02, 99%CI 2.28-11.04) and four to six early-life risk factors (OR 7.33, 99%CI 3.01-17.84). No significant associations were found at age 2 or 4 years.

Discussion

This study investigated both the impact of early-life risk factors on later overweight and obesity in Spanish children at 6 years old and their cumulative effect on the risk of becoming overweight/obese at 2, 4 and 6 years old.

Maternal BMI, paternal BMI, parental origin/ethnicity, maternal smoking during pregnancy and rapid infant weight gain were statistically significant independent factors of childhood overweigh and obesity in our investigation after adjusting for confounding factors and these early-life risk factors had an accumulative effect on overweight and obesity in children who were aged 6 years old. Particularly, there was a 7-fold increase in the risk of being overweight or obese at age 6 for children who had 4 or more risk factors, compared with children who had none.

Parental BMI and parental origin/ethnicity also confounded many of the associations studied and were strong risk factors for childhood obesity, as other investigations have revealed (Parikka et al. 2015). Parental overweight and obesity could influence the risk of obesity in their descendants due to shared genes and environmental factors within families (Whitaker et al. 1997; Williams et al. 2017). The association between parental origin/ethnicity and childhood obesity could be due genetic and cultural diversity in minority groups and those related to SES could result in differences in Energy balance-related behaviours. These groups are at higher risk of adopting an unhealthy diet, insufficient physical activity and sedentary behaviours, explaining differences found in overweight and obesity prevalence among these groups. Minorities groups (particularly, Roma/gypsies and Latin Americans) are more exposed to more vulnerabilities possibly leading to inadequate adaptation in obesogenic environments characterized by low levels of physical activity, high energy density diets and a sedentary lifestyle compared with non-vulnerable groups (Iguacel et al. 2017). Maternal educational level was used as an indicator of socioeconomic status because several studies found maternal educational level to be a reliable determinant of children's dietary behaviour and childhood obesity (van Ansem et al. 2014).

Along with our results, consistent evidence has been shown in previous studies regarding smoking during pregnancy as a risk factor for childhood overweight/obesity (Oken et al. 2008). Via intrauterine, exposure to smoke results in prenatal undernutrition. This nutritional deprivation may lead to increased nutrient achievement later and finally postnatal obesity (Oken et al. 2008). The pooled estimate from unadjusted odds ratios was higher to the adjusted estimate, suggesting maternal education and parental origin/ethnicity between smokers and non-smokers explained just partly the association.

Previous meta-analyses have showed that due to permanent alterations in metabolism excessive gestational weight gain is significantly associated with childhood overweight/obesity (Mamun et al. 2014; Tie et al. 2014). However, we did not find statically significant associations between an excessive gestational weight gain and offspring overweight, even though associations pointed to the expected directions (Sridhar et al. 2014). Part of the risk of an excessive gestational weight of childhood overweigh has been related to maternal pre-pregnancy BMI (Samura et al. 2016). Our models were adjusted for maternal pre-pregnancy BMI, which may be the result of this lack of significance as other studies have stated (Samura et al. 2016).

Concerning gestational age, we did not find any statistically significant association between gestational age and childhood obesity. In the literature, there is mixed evidence on whether gestational age is linked or not with childhood overweight/obesity (Heppe et al. 2013). Arguably infants born preterm usually compensate by engaging in rapid infant weight gain in early-life and this 'catch-up' growth is associated with an increased risk of childhood obesity. Attending previous investigations therefore, this association could be explained mainly due to early postnatal rapid infant weight gain and not directly because of gestational age (Cho and Suh 2016) but such result in not shown in our analysis.

Low and high birth weight have been associated with subsequent childhood obesity through increased leptin levels after catch-up growth during childhood and programming for lean mass respectively (Danielzik et al. 2004; Jornayvaz et al. 2016). Maternal glucose levels during pregnancy could also explain the association between birthweight and offspring of obesity. In fact, an excess of fetal insulin, due to maternal hyperglycaemia, might work as a growth hormone for the fetus and can also alter the expression of hypothalamic neurotransmitter leading to an increase in the appetite and later obesity (Guillmann 2003). However, we did not find statically significant associations between high birth weight and future overweight in children. In our analysis, only one child weighted five kg and most children who were categorized as high birthweight weighted around four kg, which could partially explain the lack of significance. Despite this lack of significance, results pointed to the expected directions and higher ORs in children who weighted more than 4 kg at birth were observed.

Birth by caesarean section has been implicated in the development of childhood obesity (Yuan et al. 2016). A recent meta-analysis has reported children born by caesarean section are at higher risk of developing obesity in childhood and this association remained significant after accounting for major confounding factors (Kuhle et al. 2015). Despite these findings, we could not find any statistically significant association between birth by caesarean section and childhood obesity.

Breastfeeding has yielded inconsistent results in the literature. Some studies have reported breastfed children have lower risk of childhood obesity than those who have not been breastfed (Yan et al. 2014) while others have stated that evidence from these studies could be influenced by confounding factors and therefore breastfeeding would not be likely to be a protective factor for childhood obesity (Lefebvre and John 2014). Our study found a raw effect between not being exclusively breastfeed in the first 4 months and future childhood overweight. Nevertheless, this effect disappeared when adjusting for maternal BMI, maternal smoking during pregnancy and education, suggesting protective effect of breastfeeding against childhood obesity could be due to confounding variables.

Early rapid infant weight gain has been reported to be a risk factor of childhood obesity (Ong and Loos 2006), which is in line with the results of our study. This factor had an independent effect on obesity risk at 6y and remained statically significant after adjusting for confounding factors and exclusive breastfeeding for at least 4 months. Specifically, rapid infant weight gain from birth to 6 months of age was the strongest predictor of later risk of childhood overweight/obesity in our study.

Finally, we examined the cumulative effect of early-life risk factors found statically significant in our study (maternal smoking during pregnancy, non-exclusive breast feeding during the first 4 months, rapid infant weight gain from birth to 6 months of age, maternal BMI >25, paternal BMI >25 and Latin American/Roma origin) in childhood overweight and obesity. Children who accumulated more risk factors had higher risk of being overweight/obese at age 6. Particularly, there was a 7-fold increase in the risk of overweight in children with 4 to 6 risk factors, 5-fold in children with 3 risk factors and 2-fold in children with 2 risk factors compared with children who had no risk factors and after adjusting for sex, age and maternal education. However, these effects were not observed at 2 and 4 years old, suggesting this tendency seems to become more pronounced over time, which have been suggested in other studies.(Robinson et al. 2015; Salsberry and Reagan 2005). These results are likely due to the combination in a same subject of both expression of genetic predisposition and being more time exposed to obesogenic environments. Furthermore, we hypothesized that children who tend to accumulate more early-life risk factors are probably more subject to socioeconomic vulnerabilities for a longer period, which may explain increased prevalence of overweight/obesity in these groups over time. Health behaviours related to obesity such as physical activity, diet or sleep and mental health can worsen due to stressful events and household dysfunction that might characterize ethnic minority and low SES groups (Iguacel et al. 2017).

Some limitations of this study should be acknowledged. Firstly, the CALINA study is not representative of Spanish population since Aragon covered a limited geographic area within the country and results might not be extrapolated to the whole population. Another limitation is reliance on self-report measures for parents (parental weight-height and education). Moreover, a selection bias cannot be precluded as there were participants (mainly children whose parents were originally from Eastern European countries, Africa and Latin America and had lower parental education) who did not complete all information required or did not continue the study at follow-up. Furthermore, some very important confounding factors such as dietary intake, income and parity were not reported and thus results must be interpreted with caution. Finally, some associations were not found to be statically significant maybe due to the small size of some groups studied (i.e. in children who weighted more than 4 kg at birth). A special strength of the study is that to our knowledge, this is the first paper investigating early-life risk factors and their accumulative effect at 2, 4 and 6 years old using a Spanish cohort in a 6 years follow-up. The prospective collection of data on a wide range of risk factors extending from pregnancy through infancy and the ability to adjust for confounding factors are also strengths of this study.

Conclusion

Parental origin/ethnicity, parental overweight and obesity, smoking during pregnancy and rapid infant weight gain were important determinants of childhood overweight/obesity. All these risk factors have cumulative effects and tend to cluster in socially patterned ways. However, these effects were not observed when children were 2 and 4 years, suggesting this tendency become more pronounced over time as children are more time exposed to these risk factors and the obesogenic environments. Therefore, the first year is critical for childhood obesity development, and its prevention. Strategies such as monitoring children with rapid infant weight gain, supporting attainment of a healthy parental weight and preventing smoking during pregnancy could be of importance for preventing childhood obesity.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Research involving human participants Ethical approval was obtained from the regional Committee of Ethics (Comité Ético de Investigación Clínica de Aragón, CEICA).

Informed consent Parents or legal guardians gave written informed consent for examinations for their children.

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Figure 1. Selection of the final study sample. Study population: children from different localities in the region of Aragon (Spain) examined from March 2009 to February 2010 and periodically re-examined 2, 4 and 6 years later.

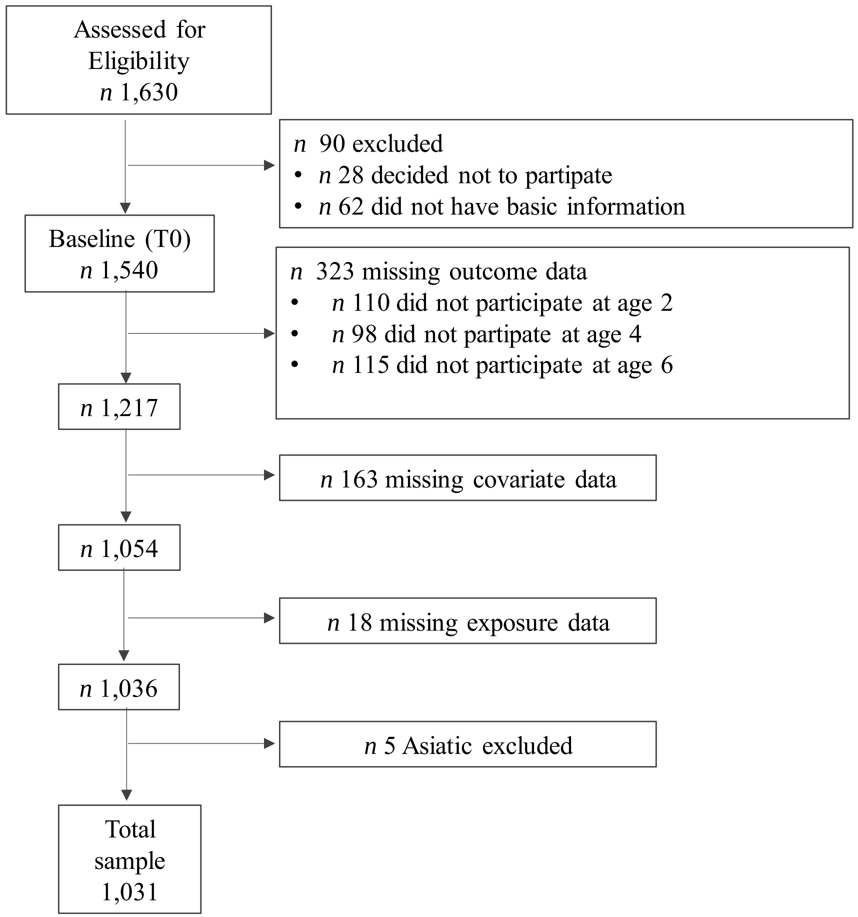

Table 1. Descriptive characteristics of the study population stratified by children's weight status (underweight/normal vs overweight/obese) at last follow-up (6 years old). Study population: children from different localities in the region of Aragon (Spain) examined from March 2009 to February 2010 and periodically re-examined 2, 4 and 6 years later.

	N (%)	EXCESS BODY WEIGHT AT 6 YEARS OLD		
		Underweight/normal weight (n 706)	**Overweight/obese** (n 325)	**P-value**
Categorical variables	N (%)	%	%	
Sex of the child				
Male	555 (53.8%)	68.1	31.9	0.783
Female	476 (46.2%)	68.9	31.1	
Maternal Education				
Missing	10 (1.0%)	80.0	20.0	**<0.001**
Low	258 (25.1%)	58.1	41.9	
Medium	349 (33.9%)	71.3	28.7	
High	414 (40.2%)	72.2	27.8	
Paternal Education				
Missing	19 (1.9%)	73.7	26.3	**<0.001**
Low	534 (32.0%)	59.7	40.3	
Medium	431 (41.8%)	71.5	28.5	
High	251 (24.3%)	74.5	25.5	
Maternal BMI				
<25 kg/m^2	754 (73.1%)	72.8	27.2	**<0.001**
25-<30 kg/m^2	534 (18.0%)	58.6	41.4	
≥30 kg/m^2	431 (8.8%)	52.7	47.3	
Paternal BMI				
<25 kg/m^2	412 (40.0%)	78.9	21.1	**<0.001**
25-<30 kg/m^2	534 (45.8%)	63.8	36.2	
≥30 kg/m^2	431 (14.3%)	54.4	45.6	
Parental origin/Ethnicity				
Spanish Roma (Gypsy)	29 (2.8%)	27.6	72.4	**<0.001**
Eastern European	40 (3.9%)	62.5	37.5	
Latin American	55 (5.3%)	47.3	52.7	
African	39 (3.8%)	69.2	30.8	
Non-gypsy Spaniard	868 (84.2%)	71.4	28.6	
Maternal smoking during pregnancy				
Yes	200 (19.4%)	59.0	41.0	**<0.001**
No	831 (80.6%)	70.8	29.2	
Gestational weight gain				
Excessive	225 (21.8%)	63.1	36.9	**0.036**
Insufficient	456 (44.2%)	65.5	27.6	

Adequate	350 (33.9%)	66.9	33.1	
Gestational age				
<37 weeks	63 (6.1%)	72.9	27.1	0.413
37-42 weeks	968 (93.9%)	70.2	29.8	
Birth weight				
<2.5 kg	66 (6.4%)	68.2	31.8	0.247
2.5-<4 kg	918 (89.0%)	69.1	30.9	
≥4 kg	47 (4.6%)	57.4	42.6	
Cesarean section				
Yes	236 (22.9%)	70.8	29.2	0.508
No	795 (77.1%)	70.5	29.5	
Exclusive breastfeeding[c]				
No	568 (55.1%)	65.4	34.5	**0.033**
Yes	463 (44.9%)	71.8	28.2	
Rapid infant weight gain				
Yes	307 (29.8%)	56.4	43.6	**<0.001**
No	717 (70.2%)	74.5	25.5	

BMI, Body Mass Index; SD, Standard Deviation.

Statistical analyses were undertaken using t-Student (for continuous variables) and chi-square tests (for categorical variables).

[a] Exclusive breastfeeding was defined as giving breast milk at the only infant food source for at least 4 months with no other liquids or food given.

Table 2. Associations between early-life risk factors and excess body weight in children at 6 years old (reference: non-overweight).

Results from the binary logistic regression models: odds ratios (OR), 99% confidence intervals (CI) are shown. Study population: children from different localities in the region of Aragon (Spain) examined from March 2009 to February 2010 and periodically re-examined 2, 4 and 6 years later.

EXCESS BODY WEIGHT (OVERWEIGHT AND OBESITY) AT 6 YEARS OLD							
Early-life risk factors			**Raw OR[a]**		**OR adjusted for confounding factors[b]**		**Confounding factors**
	N	%	**OR[a]**	**99% CI**	**OR[a]**	**99% CI**	
Parental origin/ethnicity							Maternal education, maternal BMI, breastfeeding, maternal smoking during pregnancy
Spanish Roma (gypsy)	29	2.8	**6.83**	2.28-20.45	**4.87**	2.00-11.81	
Eastern European	40	3.9	1.51	0.64-3.60	1.44	0.69-2.98	
Latin American	55	5.3	**2.79**	1.35-5.76	**3.22**	**1.79-5.77**	
African	39	3.8	1.13	0.45-2.83	1.12	**0.52-2.40**	
Non-gypsy Spaniard	868	84.2	1.00	-	**1.00**		
Maternal BMI							Maternal education, ethnicity/origin, and maternal smoking during pregnancy
Overweight	186	18.0	**1.90**	1.22-2.95	**1.91**	1.38-2.66	
Obese	91	8.8	**2.41**	1.35-4.32	**2.20**	1.41-3.42	
Normal weigh/underweight	754	73.1	1.00	-	1.00	-	
Paternal BMI							
Overweight	472	45.8	**2.12**	1.43-3.16	**2.19**	1.59-3.00	Paternal education and ethnicity/origin
Obese	147	14.3	**3.12**	1.00-2.10	**3.05**	**2.00-4.64**	
Normal weight/underweight	412	40.0	1.00	**-**	1.00	**-**	
Maternal smoking during pregnancy							Maternal education and ethnicity/origin
Yes	200	19.4	**1.68**	1.11-2.55	**1.59**	1.03-2.43	
No	831	80.6	1.00	-	1.00	-	
Gestational weight gain							Maternal BMI, maternal smoking during pregnancy, maternal education, gestational age
Excessive	225	21.8	1.18	0.80-1.73	1.13	0.65-1.97	
Insufficient	456	44.2	0.77	0.51-1.15	0.76	0.51-1.14	

	Adequate	350	33.9	1.00	-	1.00	-	
Gestational age								Maternal smoking during pregnancy and maternal education
	<37 weeks	62	6.1	0.87	0.37-2.07	0.84	0.35-2.01	
	≥37 weeks	969	93.9	1.00	-	1.00	-	
Cesarean section								Maternal BMI and gestational weight gain
	Yes	227	21.0	0.99	0.61-1.59	0.85	0.52-1.40	
	No	804	78.0	1.00	-	1.00	-	
Birth weight								Maternal smoking during pregnancy and maternal BMI
	<2.5 kg	66	19.5	1.04	0.51-2.11	0.71	0.23-2.16	
	≥4 kg	47	7.4	1.65	0.75-3.62	1.27	0.47-3.47	
	2.5-<4 kg	918	73.1	1.00	-	1.00	-	
Exclusive breastfeeding[c]								Ethnicity/origin, maternal education, maternal BMI, maternal smoking during pregnancy and breastfeeding
	No	463	45.0	**1.34**	1.01-1.82	1.20	0.82-1.75	
	Yes	568	55.0	1.00	-	1.00	-	
Rapid infant weight gain								Birth weight, breastfeeding, maternal BMI, paternal BMI, maternal education and ethnicity/origin
	Yes	217	21.1	**2.30**	1.54-3.47	**3.29**	2.00-5.41	
	No	814	78.9	1.00	-	1.00	-	

BMI, Body Mass Index; OR, Odds Ratio; CI, Confidence Interval

[a] All analyses were adjusted for sex and age at measurement.

[b] Analyses were additionally adjusted for the possible confounders of each factor.

[c] Exclusive breastfeeding was defined as giving maternal milk as the only infant food source with no other liquids or food given for at least four months.

Table 3. Associations between combined effects of early-life risk factors and excess body weight in children at 6 years old (reference: non-overweight).

Results from the binary logistic regression models: odds ratios (OR), 99% confidence intervals (CI) are shown.

Study population: children from different localities in the region of Aragon (Spain) examined from March 2009 to February 2010 and periodically re-examined 2, 4 and 6 years later.

EXCESS BODY WEIGHT (OVERWEIGHT AND OBESITY) AT 6 YEARS OLD			
	M1[a]		
Significant risk factors	**OR**	**99% CI**	**P-value**
Latin American or gypsy origin	**3.20**	1.60-6.39	<0.001
Maternal BMI ≥25 kg/m2	**1.93**	1.27-2.92	<0.001
Paternal BMI≥25 kg/m2	**2.17**	1.44-3.28	<0.001
Maternal smoking	**1.61**	1.01-2.59	0.009
Non-exclusive breastfeeding[b]	1.16	0.79-1.71	0.309
Rapid infant weight gain	**2.29**	1.54-3.42	<0.001

BMI, Body Mass Index; OR, Odds Ratio; CI, Confidence Interval; M1, model 1.

Statistically significant results are shown in bold font.

[a] All analyses were adjusted for sex and age at measurement, maternal and paternal education and all early risk factors in the respective column.

[b] Exclusive breastfeeding was defined as giving maternal milk as the only infant food source with no other liquids or food given for at least four months.

Table 4. Association between the accumulation of early life risk factors and excess body weight in children aged 2, 4 and 6 years old (reference: non-overweight) for the three models *. Results from the binary logistic regression models: odds ratios (OR) and 99% confidence intervals (CI) are shown. Study population: children from different localities in the region of Aragon (Spain) examined from March 2009 to February 2010 and periodically re-examined 2, 4 and 6 years later.

	EXCESS BODY WEIGHT (OVERWEIGHT AND OBESITY) AT 6 YEARS OLD									
Number of early-life risk factors [a]		**At 2 y** [b]			**At 4 y** [b]			**At 6 y** [b]		
	N	**OR**	**99% CI**	**P-value**	**OR**	**99% CI**	**P-value**	**OR**	**99% CI**	**P-value**
4-6	99	0.92	0.29-2.86	0.852	1.39	0.53-3.67	0.378	**7.33**	3.01-17.84	<0.001
3	235	0.79	0.30-2.08	0.540	1.08	0.47-2.50	0.801	**5.02**	2.28-11.04	<0.001
2	325	1.40	0.58-3.39	0.322	1.23	0.56-2.72	0.488	**2.72**	1.26-5.88	0.001
1	265	0.73	0.28-1.87	0.390	0.85	0.37-1.93	0.603	0.97	0.42-2.23	0.920
0	107	1.00			1.00			1.00		

Statistically significant results are shown in bold font.

[a] The total number of early-life risk factors was calculated by adding up the numbers of early-life risk factors the child was exposed to: maternal smoking during pregnancy; not being exclusively breastfeed during the first 4 months, rapid infant weight gain, maternal BMI >25 BMI, paternal BMI >25 BMI, and Latin American or gypsy origin. The total number of early-life risk factors ranged from 0 (the child had none of the early-life risk factors) to 6 (the child had all six early-life risk factors) and was divided into five categories (four to six risk factors, three risk factors, two risk factors, one risk factor and no risk factors).

[b] Models were adjusted for sex and age at measurement, maternal and paternal education.

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The authors' contributions were as follows: I. I carried out the statistical analysis and drafted the manuscript along with G. R., L. E., J. F.-A., I. L., I. I., L. A. M., G. R., and M. P. S., collected the data, supervised the data procedure and read and critically reviewed the manuscript.

GENUD Research Group
Growth, Exercise, NUtrition and Development
Universidad Zaragoza

Dear Editor-in-chief,

Enclosed you will find a revision of our manuscript, **"Early-life risk factors and their cumulative effect as predictors of overweight in Spanish children"**. Changes in the manuscript have been highlighted using a red font.

This manuscript contains material that is original and not previously published in text or on the Internet, nor is it being considered elsewhere until a decision is made as to its acceptability by the International Journal of Public Health Editorial Review Board.

Isabel Iguacel Azorín

GENUD (Growth, Exercise, NUtrition and Development) Research Group, Faculty of Health Sciences; University of Zaragoza, Edificio del SAI, C/Pedro Cerbuna s/n, 50009 Zaragoza, Spain

Editorial comments:

Although statement on regional ethics committee approval for this study is provided, the full name of the regional ethics committee that approved the study should be provided in the manuscript.

Answer: Thank you. We have included in the manuscript the full name of the regional ethics committee:

"Ethical approval was obtained from the regional Committee of Ethics (Comité Ético de Investigación Clínica de Aragón, CEICA)".

Also, we observed that the email address of the corresponding author in the manuscript is different from that mentioned in the editorial submission System, please provide the same email address in the electronic system and in the manuscript.

Answer: Thank you. The same email address (iguacel@unizar.es) has been included.

Electronic supplementary material: please provide the journal name and article title on top.

Answer: Thank you. The journal name and article title on top have been included in electronic supplementary material.