Hormones and Behavior 126 (2020) 104842

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Leptin at birth and at age 7 in relation to appetitive behaviors at age 7 and age 10

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ARTICLE INFO

Keywords:
Children
Leptin
Cohort studies
Feeding behaviors
Pediatric obesity/overweight

ABSTRACT

Leptin contributes to the control of food intake and energy balance. However, its association with appetitive behaviors during childhood is not well understood. We aimed to investigate the association between leptin, assessed at birth and at 7 years of age (y), and appetitive behaviors assessed at 7 and 10 y. Children from a Portuguese cohort with assessment of leptin levels at birth from umbilical cord blood (n = 645) and at 7 y from venous blood samples (n = 587), were included. The Children's Eating Behavior Questionnaire assessed appetitive behaviors at 7 and 10 y. Weight and height were measured at 7 and 10 y to derive BMI z-scores (BMIz). A series of Generalized Linear Models tested relationships between leptin and appetitive behaviors, adjusting for potential confounders (maternal age, education, pre-pregnancy BMI, smoking during pregnancy, child physical activity and child BMIz), and interaction terms for child sex and child BMIz. At 7 y, 116 boys and 118 girls were classified as having overweight/obesity, and these children had higher leptin levels. Cross-sectional analyses using the 7 y data produced the strongest results. Higher leptin at 7 y was significantly associated with lower scores on Satiety Responsiveness, Food Fussiness and Slowness in Eating, and higher scores on Food Responsiveness, Enjoyment of Food and Emotional Overeating at 7 y. Only the association with Emotional Overeating remained when adjusting for child BMIz. Significant interaction effects between child sex and leptin were found for appetite at 7 y, such that higher leptin was associated with higher Food Responsiveness ($p < 0.001$) and lower Slowness in Eating ($p < 0.001$) to a greater extent among boys. Umbilical cord blood leptin was not associated with appetitive behaviors at 7 or 10 y. Our results show that leptin levels are positively associated with food approach and negatively with food avoidant behaviors. Associations were more consistent in cross-sectional analyses (at 7 y), were largely dependent on child weight, and tended to be stronger among boys. Our findings support a role for leptin in affecting appetite, with potential consequences for current weight status and future weight gain.

1. Introduction

The worldwide epidemic of obesity and overweight (World Health Organization, 2018) demands greater knowledge of obesity-associated pathophysiological mechanisms that could drive excess intake. Adipose tissue is now understood to be not solely an energy reservoir, but a complex endocrine organ (Galic et al., 2010), and adipocytokines and their dysfunction (e.g. lack of, or resistance to, an adipose tissue-derived hormone) have been recognized as an etiological factor in obesity-associated disorders (Cao, 2014). Leptin is an adipocytokine playing a role in neuroendocrine function and energy balance (Farr et al., 2015). Primarily, leptin acts in the hypothalamus to coordinate energy homeostasis. Circulating leptin levels reflect the amount of stored body fat, with decreases driven by inadequate energy stores (e.g. during starvation or genetic leptin deficiency). This leptin response acts to enhance appetite and decreasing energy expenditure (Bates and Myers, 2003). In contrast, higher levels of leptin ('hyperleptinaemia') decrease the drive to eat and enable energy utilization (Bates and Myers, 2003). Hyperleptinaemia has been robustly linked to excessive weight (Jois et al., 2015; Mi et al., 2010; Nappo et al., 2017), and can be explained by higher fat mass, since leptin release per gram of adipose tissue is, on average, two times greater in subjects with obesity compared to their lean peers (Galic et al., 2010). However, leptin does not act to decrease body weight in individuals with overweight, suggesting a leptin resistance phenomenon (Jois et al., 2015; Nappo et al., 2017). Overweight and accompanying leptin resistance is frequently the

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https://doi.org/10.1016/j.yhbeh.2020.104842
Received 16 December 2019; Received in revised form 5 August 2020; Accepted 14 August 2020
Available online 09 September 2020

consequence of an overconsumption of highly palatable foods, which may, in turn, be driven by hedonic eating (i.e. eating based on pleasure) (Murray et al., 2014).

Since leptin influences food intake (Kanoski et al., 2011) and plays a role in reward, motivational and cognitive processes (Farr et al., 2015), it may also have a role in driving eating behaviors, and in determining the development of these behaviors in childhood, i.e. leptin may be an etiological and maintenance factor for obesogenic eating behavior as well as an end-product of overeating and ensuing overweight. Previous studies have linked food approach behaviors assessed using the Children's Eating Behavior Questionnaire (CEBQ) (Wardle et al., 2001) (e.g. Enjoyment of Food, Food Responsiveness and Emotional Overeating) to increased weight gain among children (Derks et al., 2018; van Jaarsveld et al., 2011) and food avoidant behaviors (e.g. Food Fussiness and Picky Eating) to lower weight (Berger et al., 2016; Rahill et al., 2019). Further, one study of a small sample of children with obesity in Chile (n = 134) using the CEBQ and the Three-Factor Eating Questionnaire (de Lauzon et al., 2004), reported sex-specific relationships of polymorphisms in the leptin gene and its receptor with food approach and food avoidant behaviors (Valladares et al., 2015). Another study aiming to examine changes in eating behaviors and plasma leptin concentrations in a sample of Canadian children with overweight/obesity (n = 78) following a 1-year intervention, found a positive correlation between plasma leptin and the CEBQ sub-scale Desire to Drink (Cohen et al., 2018). However, to our knowledge, there is a lack of studies that investigate, using a longitudinal design, if childhood appetitive behaviors are associated with or predicted by leptin levels at birth or during childhood. There is also little understanding of how sex might impact this relationship, given that females have higher body fat and therefore higher leptin levels (Christen et al., 2018; Jois et al., 2015), with potential effects on appetite.

The aim of this study was to investigate the association between leptin levels, assessed at birth and at 7 years, and appetitive behaviors at 7 and 10 years of age. We predicted that (i) higher leptin levels at 7 years would be associated with higher scores on the food approach sub-scales *Enjoyment of Food*, *Food Responsiveness*, *Emotional Overeating* and *Desire to Drink* at 7 and 10 years of age; (ii) higher leptin levels at 7 years would be associated with lower scores on the food avoidant behavior sub-scales *Satiety Responsiveness*, *Food Fussiness*, *Slowness in Eating* and *Emotional Undereating* at 7 and 10 years of age; (iii) leptin levels at birth, measured from the umbilical cord, would be associated with higher food approach behavior sub-scale scores and lower food avoidant behavior sub-scale scores at 7 and 10 years of age. We also anticipated that results would be modified by sex and BMI z-score such that we would see stronger associations between leptin and appetitive behaviors among girls, and among heavier children.

2. Material and methods

2.1. Participants

This study included children from Generation XXI, an ongoing prospective population-based birth cohort from Northern Portugal. Recruitment occurred between April 2005 and August 2006, at all public maternity wards of the metropolitan area of Porto; they were responsible, at enrolment, for 91.6% of the deliveries in the whole catchment population. Of all eligible mothers, 91% agreed to participate (8495 mothers and 8647 children at baseline). Further details about data collection are described elsewhere (Alves et al., 2012; Larsen et al., 2013).

Follow-up assessments were undertaken at ages 4 years old (y) (April 2009–August 2011), 7 y (April 2012–March 2014) and 10 y (July 2015–January 2017), with a participation rate of 86%, 80% and 76%, respectively. The present study included variables from the baseline (birth), 7 y and 10 y follow-ups. Among those who had umbilical cord blood collected at birth and a physical examination at 7 years of age (excluding twins and follow-up losses), adipocytokines, including leptin, were assessed in the umbilical cord blood (n = 899 children) and at 7 y, using a venous blood sample from the child (n = 729). Children were excluded if they (1) did not have information on eating behaviors at 7 and 10 y (n = 254 and n = 115, respectively), and (2) did not have information on variables of interest (n = 27 among children with blood measurement at 7 y), resulting in a final sample of 645 with blood from the umbilical cord and 587 with a venous blood sample at 7 years of age.

Comparisons of characteristics of the study sample with measures of venous blood at 7 y (n = 587) with the remaining sample at baseline (n = 8061), revealed that non-participants were slightly less heavy at birth (mean ± SD 3141.20 g ± 563.38 compared with 3228.72 g ± 442.57, $p < 0.001$) and their mothers were less educated (mean ± SD 10.4 ± 4.25 completed years of education compared to 11.5 ± 4.22, $p < 0.001$) and younger (mean ± SD 28.9 y ± 5.62 compared to 30.1 y ± 5.20, $p < 0.001$). There were no differences on maternal Body Mass Index (BMI) at baseline, or on child sex or BMI z-score (BMIz) at 7 y. The magnitudes of the differences based on Cohen's *d* effect size values, were not high (0.26 for maternal education and 0.16 for birth weight), suggesting that the differences we observed were driven by the large sample size.

2.2. Measures

All newborns were weighed at birth and birth weight was extracted from clinical records. Additional data, such as maternal age, education, pre-pregnancy BMI, gestational weight gain, and whether the mother smoked during pregnancy, were obtained by face-to-face interviews conducted by trained researchers within 24 to 72 h of delivery.

At birth, a cord blood sample (approx. 8.5 mL), and at the 7 y follow-up, a fasting venous blood sample (approx. 8 mL), was drawn using standard procedures for the determination of the leptin levels. Cord blood and venous blood samples were sent to the laboratory for analyses within a maximum of 2 h after collection, kept refrigerated until centrifugation at 3500 rpm within 15 min, and stored at −80 °C for later analysis. Leptin levels in plasma were determined by enzyme-linked immunosorbent assay (Mercodia Leptin ELISA kit, Mercodia AB, Uppsala, Sweden).

At the same 7 y visit, height and weight were measured by trained researchers, following standard procedures. BMI was computed for each child, and age- and sex- specific BMIz scores using World Health Organization (WHO) reference data (de Onis et al., 2007) were calculated. In order to assess differences in leptin levels by child weight status, under/normal weight and overweight/obese categories were created; children with BMIz < +1SD were classified as under/normal weight (Uw/Nw) and BMIz ≥ +1SD were classified as having overweight or obesity (OW/Ob). This classification was based on the sample distribution and is used for sample descriptives. Child BMIz as a continuous variable was used for the association and interaction analyses. In addition, physical activity was estimated by asking 'How long does [name of child] spend, on average per day, in leisure-time activities (running, playing ball, cycling) during the week (Monday to Friday)?' and 'How long does [name of child] spend, on average per day, in leisure-time activities (running, playing ball, cycling) during the weekend (Saturday to Sunday)?', with answers given in average time, per week/weekend, in hours. Weighted physical activity time was calculated using the following equation: ((time of physical activity in the weekend * 2) + (time of physical activity in weekdays * 5)) / 7, representing the mean daily time in hours spent on physical activities over the whole week.

2.3. Appetitive behaviors

Appetitive behaviors were assessed at 7 and 10 y using the CEBQ, which was validated in a Portuguese sample of school-aged children

from Generation XXI (Albuquerque et al., 2017). Parents or main caregivers were asked to respond to the 35-item questionnaire, which assesses eight sub-scales: *Satiety Responsiveness* (CEBQ-SR - 5 items, e.g. *My child leaves food on his/her plate at the end of a meal*), *Slowness in Eating* (CEBQ-SE - 4 items, e.g. *My child eats slowly*), *Food Fussiness* (CEBQ-FF - 6 items, e.g. *My child is difficult to please with meals*), *Emotional Undereating* (CEBQ-EUE - 4 items, e.g. *My child eats less when s/he is upset*), *Food Responsiveness* (CEBQ-FR - 5 items, e.g. *If allowed to, my child would eat too much*), *Enjoyment of Food* (CEBQ-EF - 4 items, e.g. *My child loves food*), *Desire to Drink* (CEBQ-DD - 3 items, e.g. *My child is always asking for a drink*) and *Emotional Overeating* (CEBQ-EOE - 4 items, e.g. *My child eats more when annoyed*). The sub-scales Satiety Responsiveness, Slowness in Eating, Food Fussiness and Emotional Undereating were considered 'food avoidant' behaviors, and the remaining sub-scales, namely Food Responsiveness, Enjoyment of Food, Desire to Drink and Emotional Overeating were considered 'food approach' behaviors, as previously described (Webber et al., 2009). Answers were given using a 5-point Likert scale, ranging from 1 - 'never' to 5 - 'always' such that the higher the score, the more frequent the eating behavior. In accordance with the original scale, five of the items were reverse-scored. For questionnaires that were missing < 50% of data items (around 3% of sample), sub-scale scores were calculated by replacing missing items with the mean of the present items. Albuquerque et al. (2017) reported good internal consistency for the validated CEBQ with Cronbach's α coefficients ranging from 0.74 to 0.85 at 7 y (Albuquerque et al., 2017), and from 0.76 to 0.84 at 10 y (Costa et al., 2020). Adequate internal consistency was also observed in the current population, with Cronbach's α coefficients ranging from 0.78 to 0.84 at 7 y and from 0.76 to 0.88 at 10 y (data not shown).

2.4. Statistical analysis

Continuous variables were expressed as mean and standard deviations (M (SD)) or median and interquartile ranges (Md (IQR)). Effect sizes were also estimated, using Cohen's *d* and eta squared for parametric and non-parametric comparisons, and Cramérs V for categorical variables (Cohen, 1988; Husted et al., 2000; Lenhard and Lenhard, 2016). Cohen's *d* effect sizes of ≥0.8 were considered large, 0.5–0.7 intermediate, 0.2–0.4 small and < 0.2 as indicating no effect. For eta squared, effect sizes of ≥0.140 were considered large, 0.060–0.110 intermediate, 0.010–0.039 small and < 0.003 as indicating no effect (Cohen, 1988). Normality was evaluated for each variable using the Kolmogorov–Smirnov test and leptin values were compared using Student's *t*-tests or Mann-Whitney *U* tests, stratified by child sex and weight status at 7 y. Cross-sectional associations between leptin levels and CEBQ sub-scale scores at 7 y, and prospective associations between leptin levels at 7 y and CEBQ sub-scale scores at 10 y were tested. Additionally, we tested the associations between cord blood leptin levels and appetitive behaviors at 7 and 10 y. For all analyses, crude and adjusted linear regression coefficients (β) and 95% confidence intervals (95% CI) were computed using Generalized Linear Models (GLzM). First, models were adjusted for potential confounders of relationships between leptin levels and appetitive behaviors, i.e. variables with known influences on both CEBQ sub-scale scores and adiposity (and therefore leptin levels). These were maternal age and education (e.g. Albuquerque et al., 2017; Boeke et al., 2013; Schrempft et al., 2016), pre-pregnancy BMI (e.g. Boeke et al., 2013; Kolko et al., 2018; Mantzoros et al., 2009), smoking during pregnancy (e.g. Boeke et al., 2013; Rückinger et al., 2010), and child mean daily time of physical activity at 7 y (e.g. Cohen et al., 2018; Plonka et al., 2011). In the 10 y analyses, models additionally included the child's respective appetitive trait at 7 y. To investigate whether CEBQ-leptin associations were driven by or independent of established relationships between CEBQ and body weight (Berger et al., 2016; Derks et al., 2018; Rahill et al., 2019), Model 2 was further adjusted for child BMIz at the respective age (i.e. BMIz at 7 y and at 10 y). Next, in order to assess potential modification effects of child weight (due to possible leptin resistance among children with overweight or obesity), and child sex (due to differences in body composition between boys and girls), two-way interaction terms (leptin levels × BMIz at each age (Model 3), and leptin levels × child sex (female as reference category) (Model 4a), were added to the previously adjusted model (i.e. Model 2)). In addition, since tests of collinearity (test of tolerance and Durbin-Watson test) indicated a tendency toward collinearity between BMIz and leptin levels, we also repeated Model 4 without controlling for BMIz (Model 4b). Three-way interactions (leptin levels × BMIz at each age × child sex) were also investigated, and added to Model 2. All variables, with the exception of the outcome variables, were centered at their means to reduce multicollinearity and ease interpretation in the interaction analyses (Aiken and West, 1991; Cohen et al., 2003). Statistical significance was set at a two-tailed p-value < 0.05. All statistical analyses were carried out using IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. released 2017.

2.5. Ethics

Generation XXI was approved by the University of Porto Medical School/S. João Hospital Centre Ethics Committee and by the Portuguese Data Protection Authority. All the phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki. Accordingly, a written informed consent from the parents (or legal substitute) and an oral consent from the children were obtained at each evaluation.

3. Results

Table 1 shows comparisons of anthropometric and behavioral characteristics, at 7 and 10 years, between children with and without overweight/obesity classified according to 7 y data, for each sex. Maternal pre-pregnancy BMI was significantly higher, in both sexes, among those children with overweight/obesity. Boys and girls with overweight/obesity showed higher leptin at 7 y than under/normal weight boys and girls, corresponding to large effect sizes (eta squared > 0.5). Girls in both weight categories also showed higher levels of circulating leptin compared to boys in both weight categories (p < 0.001 according to Mann-Whitney testing). At both 7 and 10 y, boys and girls with overweight/obesity showed lower mean CEBQ scores on food avoidant sub-scales (Satiety Responsiveness, Slowness in Eating and Food Fussiness), and higher mean CEBQ scores on food approach sub-scales (Food Responsiveness, Enjoyment of Food, Emotional Overeating), compared to under/normal weight boys and girls. Among girls only, those with overweight/obesity showed lower Emotional Undereating at 7 y (Uw/Nw: M = 2.63, SD = 0.74 vs. Ow/Ob: 2.31, 0.72 (p < 0.001), and at 10 y (Uw/Nw: M = 2.50, SD = 0.72 vs. Ow/Ob: 2.33, 0.72 (p = 0.045)). The largest effect sizes for weight group comparisons of CEBQ scores were for Food Responsiveness in boys at 7 y (Cohen's *d* = 0.80, Uw/Nw: M = 1.86, SD = 0.58 vs. Ow/Ob: 2.43, 0.87 (p < 0.001)) and Satiety Responsiveness in girls at 7 y (Cohen's *d* = 0.73, Uw/Nw: M = 2.91, SD = 0.63 vs. Ow/Ob: 2.47, 0.54 (p < 0.001)). At 10 y, we observed larger effect sizes for Enjoyment of Food among boys (Cohen's *d* = 0.67, Uw/Nw: M = 3.03, SD = 0.79 vs. Ow/Ob: 3.54, 0.71 (p < 0.001)), and for Food Responsiveness among girls (Cohen's *d* = 0.89, Uw/Nw: M = 1.93, SD = 0.62 vs. Ow/Ob: 2.62, 0.96 (p < 0.001)). Children with overweight/obesity at 7 y had a higher BMIz than those with under/normal weight at 10 y, indicating stability in weight status; this was also evident from correlations of BMIz values at 7 and 10y (Pearsons r = 0.88).

Table 2 shows cross-sectional relationships between circulating leptin and appetitive behaviors at 7 y old. Since crude results were similar to those with the first set of adjustments for maternal characteristics and child's physical activity (Model 1), we present just the adjusted results in the table; Model 2 was adjusted for maternal education,

Table 1
Maternal and child characteristics by weight status (with and without overweight/obesity) at 7 years old, stratified by sex (n = 587).

	Boys			Girls		
	Uw/Nw (< +1SD) (n = 173)	OW/Ob (≥ + 1 SD) (n = 116)	*d*	Uw/Nw (< +1SD) (n = 180)	OW/Ob (≥ + 1 SD) (n = 118)	*d*
	Md (IQR)					
Maternal and child variables at baseline						
Mother's age (y)	**31.0 (6.0)**	**30.0 (6.0)***	0.01[b]	30.0 (7.0)	30.0 (9.0)	0.01[b]
Gestational weight gain (kg)	13.0 (6.0)	14.0 (30.0)	0.01[b]	13.0 (6.0)	15.0 (7.0)	0.01[b]
Pre-pregnancy BMI (kg/m^2)	**22.4 (4.6)**	**24.3 (5.7)****	0.10[b]	**22.3 (4.6)**	**24.4 (5.1)***	0.10[b]
Mother education (y)	12.0 (17.0)	12.0 (16.0)	0.01[b]	**12.0 (7.0)**	**9.0 (5.0)***	0.28[b]
Smoking during pregnancy (yes) (n (%))	31 (18.0)	28 (24.6)	0.079[a]	33 (18.4)	30 (25.9)	0.089[a]
Birth weight at baseline (g) – M (SD)	**3249.7 (438.0)**	**3354.3 (428.3)***	0.26	**3099.7 (474.5)**	**3285.9 (357.8)****	0.43
Gestational age at birth (weeks)	39.0 (2.0)	39.0 (8.0)	0.03[b]	39.0 (2.0)	39.0 (5.0)	0.02[b]
Intrauterine growth (n (%))						
SGA	21 (12.1)	9 (3.1)	0.077[a]	33 (18.3)	10 (8.5)	0.138[a]
AGA	148 (85.5)	103 (88.8)		140 (77.8)	102 (86.4)	
LGA	4 (2.3)	4 (3.4)		7 (3.9)	6 (5.1)	
Umbilical cord blood leptin (ng/mL)[c]	11.8 (9.78)	11.3 (11.3)	0.00	17.9 (16.6)	19.4 (18.0)	0.00
Child anthropometric and metabolic characteristics at 7 y						
BMI z-score – M (SD)	**−0.01 (0.67)**	**1.85 (0.70)****	2.72	**−0.03 (0.69)**	**1.87 (0.68)****	2.77
Leptin (ng/mL)	**2.56 (1.75)**	**5.60 (5.34)****	0.55[b]	**3.48 (2.85)**	**9.50 (9.0)****	0.65[b]
Child eating behaviors at 7 y - M (SD) [CEBQ ranges from 1 ('never') to 5 ('always')]						
CEBQ-SR	**2.85 (0.67)**	**2.44 (0.56)****	0.65	**2.91 (0.63)**	**2.47 (0.54)****	0.73
CEBQ-SE	**3.06 (0.88)**	**2.47 (0.78)****	0.69	**3.17 (0.83)**	**2.61 (0.73)****	0.71
CEBQ-FF	**3.03 (0.78)**	**2.83 (0.74)***	0.26	**3.04 (0.72)**	**2.77 (0.72)***	0.38
CEBQ-EUE	2.48 (0.73)	2.38 (0.74)	0.14	**2.63 (0.74)**	**2.31 (0.72)****	0.43
CEBQ-FR	**1.86 (0.58)**	**2.43 (0.87)****	0.80	**1.87 (0.55)**	**2.37 (0.86)****	0.72
CEBQ-DD	2.31 (0.87)	2.33 (0.82)	0.02	2.34 (0.82)	2.32 (0.72)	0.00
CEBQ-EF	**2.86 (0.73)**	**3.34 (0.73)****	0.66	**2.89 (0.69)**	**3.39 (0.76)****	0.70
CEBQ-EOE	**1.80 (0.57)**	**1.95 (0.64)***	0.24	**1.77 (0.51)**	**1.93 (0.74)***	0.26
Child anthropometric characteristics at 10 y						
BMI z-score - M (SD)	**0.19 (0.97)**	**1.86 (0.75)****	1.87	**−0.06 (0.93)**	**1.79 (0.69)****	2.19
Child eating behaviors at 10 y - M (SD) [CEBQ ranges from 1 ('never') to 5 ('always')]						
CEBQ-SR	**2.63 (0.67)**	**2.42 (0.61)***	0.32	**2.79 (0.67)**	**2.35 (0.53)****	0.71
CEBQ-SE	**2.70 (0.93)**	**2.29 (0.79)****	0.46	**2.94 (0.79)**	**2.45 (0.77)****	0.63
CEBQ-FF	**3.07 (0.83)**	**2.84 (0.73)***	0.29	**2.96 (0.75)**	**2.76 (0.78)***	0.25
CEBQ-EUE	2.39 (0.70)	2.42 (0.72)	0.05	**2.50 (0.72)**	**2.33 (0.72)***	0.24
CEBQ-FR	**2.04 (0.71)**	**2.50 (0.84)****	0.60	**1.93 (0.62)**	**2.62 (0.96)****	0.89
CEBQ-DD	2.16 (0.73)	2.26 (0.74)	0.15	2.08 (0.70)	2.18 (0.71)	0.15
CEBQ-EF	**3.03 (0.79)**	**3.54 (0.71)****	0.67	**3.01 (0.64)**	**3.53 (0.79)****	0.74
CEBQ-EOE	**1.88 (0.62)**	**2.14 (0.75)***	0.39	**1.87 (0.52)**	**2.14 (0.71)****	0.44

M (SD): means and standard deviations. Md (IQR): medians and inter-quartile ranges. *d*: Cohen's d effect size. BMI: Body Mass Index; CEBQ: Children's Eating Behavior Questionnaire; CEBQ-SR: Satiety Responsiveness; CEBQ-SE: Slowness in Eating; CEBQ-FF: Food Fussiness; CEBQ-EUE: Emotional Undereating; CEBQ-FR: Food Responsiveness; CEBQ-DD: Desire to Drink; CEBQ-EF: Enjoyment of Food; CEBQ-EOE: Emotional Overeating; SGA: small-for-gestational age; AGA: adequate-for-gestational age; LGA: large-for-gestational age. Child eating behaviors range from 1 - 'never' to 5 - 'always'. Bold indicates values significantly different between weight groups: ** = ≤0.001; * = ≤0.05 p-value, based on independent sample t-test or Mann-Whitney *U* test for two samples (Uw/Nw vs. OW/Ob children).

[a] Cramér's V effect size.

[b] Eta squared effect size.

[c] n = 645.

maternal age, pre-pregnancy BMI, smoking during pregnancy, and child mean daily time of physical activity at 7 y, and also included child BMIz at 7 y as a covariate. According to the first multivariate model, higher circulating leptin levels at 7 y were associated with lower scores on sub-scales assessing food avoidant behaviors (Satiety Responsiveness, Slowness in Eating and Food Fussiness) and higher scores on those assessing food approach behaviors (Food Responsiveness, Enjoyment of Food and Emotional Overeating). In other words, we observed that, for example, a one-unit increase in leptin levels (in ng/dL) was associated with a decrease of 0.02 on the Satiety Responsiveness sub-scale (possible range 1–5). We did not find any significant relationship between leptin levels and the sub-scales Emotional Undereating and Desire to Drink (Table 2).

After further controlling for child BMIz at 7 y (Model 2), the association between leptin and Emotional Overeating was maintained while the remaining sub-scales lost their statistical significance.

Further analyses including 2-way interaction terms demonstrated no significant interactions between leptin levels and BMIz (Model 3). Since a tendency toward BMIz and leptin levels at 7 y tended toward collinearity, we performed analyses including BMIz and also excluding this variable from the models. In Model 4a, which included child BMIz, leptin was associated with lower Slowness in Eating at 7 y (β(95% CI) −0.03 (−0.06; −0.01)), however the interaction term leptin × sex was non-significant. In Model 4b, which did not include child BMIz, two significant interactions between leptin levels and sex emerged, such that higher leptin levels were associated with lower Slowness in Eating (β(95% CI) −0.07 (−0.09; −0.04)) and higher Food Responsiveness (β(95% CI) 0.05 (0.04; 0.07)) to a greater extent among boys. Because variables are centered to their means, we can interpret that a one-unit increase in leptin levels (in ng/dL) corresponds on average to a decrease of 0.07 on Slowness in Eating and an average increase of 0.05 of Food Responsiveness. To further examine these relationships, scatterplots for

Table 2
Generalized linear models assessing cross-sectional relationships between leptin and appetitive behaviors at 7 years old.

	Food avoidant behaviors				Food approach behaviors			
	CEBQ-SR	CEBQ-SE	CEBQ-FF	CEBQ-EUE	CEBQ-FR	CEBQ-EF	CEBQ-DD	CEBQ-EOE
	β (95% CI)							
Model 1	**−0.02 (−0.03; −0.01)**	**−0.04 (−0.05; −0.03)**	**−0.01 (−0.02; 0.00)**	−0.01 (−0.02; 0.00)	**0.03 (0.02; 0.04)**	**0.03 (0.02; 0.04)**	0.00 (−0.01; 0.01)	**0.02 (0.01; 0.02)**
Model 2	0.00 (−0.01; 0.01)	−0.01 (−0.02; 0.00)	0.00 (−0.01; 0.01)	0.01 (−0.01; 0.02)	0.00 (−0.01; 0.01)	0.00 (−0.01; 0.02)	0.00 (−0.01; 0.02)	**0.01 (0.00; 0.02)**
Model 3	0.00 (−0.02; 0.02)	0.01 (−0.02; 0.03)	0.00 (−0.02; 0.02)	−0.02 (−0.03; 0.00)	−0.01 (−0.03; 0.01)	−0.01 (−0.03; 0.01)	−0.01 (−0.04; 0.01)	0.01 (−0.01; 0.02)
Leptin × BMIz interaction	0.00 (−0.01; 0.01)	−0.01 (−0.02; 0.00)	0.00 (−0.01; 0.01)	0.00 (−0.01; 0.01)	0.01 (0.00; 0.01)	0.01 (0.00; 0.02)	0.01 (0.00; 0.02)	0.00 (0.00; 0.01)
Model 4a	−0.01 (−0.03; 0.01)	**−0.03 (−0.06; −0.01)**	0.00 (−0.02; 0.03)	0.01 (−0.02; 0.03)	0.02 (0.00; 0.04)	0.01 (−0.01; 0.03)	0.01 (−0.01; 0.04)	0.02 (0.00; 0.04)
Leptin × Sex interaction	0.01 (−0.01; 0.02)	0.02 (0.00; 0.05)	−0.01 (−0.03; 0.02)	−0.01 (−0.03; 0.02)	−0.02 (−0.04; 0.00)	−0.01 (−0.03; 0.01)	−0.01 (−0.04; 0.01)	−0.01 (−0.02; 0.01)
Model 4b	**−0.04 (−0.05; −0.02)**	**−0.07 (−0.09; −0.04)**	−0.01 (−0.03; 0.01)	−0.01 (−0.03; 0.01)	**0.05 (0.04; 0.07)**	**0.05 (0.03; 0.07)**	0.01 (−0.01; 0.03)	**0.02 (0.01; 0.04)**
Leptin × Sex interaction	0.01 (−0.01; 0.03)	**0.03 (0.01; 0.06)**	0.00 (−0.03; 0.03)	0.00 (−0.02; 0.02)	**−0.03 (−0.05; −0.01)**	−0.02 (−0.04; 0.00)	−0.01 (−0.04; 0.01)	−0.01 (−0.03; 0.01)

CI: confidence intervals; CEBQ: Children's Eating Behavior Questionnaire. CEBQ-SR: Satiety Responsiveness; CEBQ-SE: Slowness in Eating; CEBQ-FF: Food Fussiness; CEBQ-EUE: Emotional Undereating; CEBQ-FR: Food Responsiveness; CEBQ-EF: Enjoyment of Food; CEBQ-DD: Desire to Drink; CEBQ-EOE: Emotional Overeating. Scores range from 1 - 'never' to 5 - 'always'. Model 1 adjusted for maternal education, maternal age, pre-pregnancy BMI, smoking during pregnancy, and child mean daily time of physical activity at 7 y. Model 2 adjusted for Model 1 plus child BMIz at 7 y. Model 3 adjusted for Model 2 plus interaction term Leptin levels at 7 y × BMIz. Model 4a adjusted for Model 2 plus interaction term Leptin levels at 7 y × child sex (female as reference category). Model 4b adjusted for Model 1 plus interaction term Leptin levels at 7 y × child sex (female as reference category). **Bold** indicates significant main effect of leptin levels or interaction terms ($p < 0.05$).

each sex were created, and are presented graphically in Fig. 1. Three-way interaction terms (leptin × BMIz × sex) did not reach statistical significance for any of the sub-scales (data not shown).

Prospective relationships between leptin levels at 7 y and appetitive behaviors at 10 y are described in Table 3. As for the cross-sectional analyses, crude results were similar to the adjusted results and we therefore present only adjusted relationships of leptin levels at 7 y with appetitive behaviors at 10 y. Higher leptin levels at 7 y were associated with lower scores on the food avoidant sub-scale Satiety Responsiveness at 10 y. For example, a one-point increase of leptin (in ng/dL) at 7 y was associated with a decrease of 0.01 points on Satiety Responsiveness at 10 y. No relationships were apparent for Food Fussiness, Slowness in Eating and Emotional Undereating. Higher leptin levels at 7 y were additionally associated with higher scores on the food approach sub-scales Food Responsiveness, Enjoyment of Food and Emotional Overeating at 10 y, showing a similar pattern to the cross-sectional associations at 7 y. After further adjusting for child BMIz at 10 y (Model 2), none of these effects retained statistical significance. The models that included the two-way interactions between leptin and BMIz (Model 3) and between leptin and child sex (Models 4a and b), and the three-way interaction leptin × BMIz × sex (data not shown), were not significant for any of the sub-scales assessed at 10 y.

Using umbilical cord blood, we analyzed associations of children's in utero leptin levels with later appetitive behaviors (at 7 y and 10 y). No significant relationships between cord blood leptin and appetitive behaviors later in childhood were detected (data not shown).

4. Discussion

The present study aimed to examine the relationship between leptin levels, assessed at birth and 7 y, and appetitive behaviors reported by parents at 7 and 10 years of age.

First, in agreement with previous studies of school-aged children (Cohen et al., 2018; Gil-Campos et al., 2010; Madeira et al., 2016), we found that boys and girls with overweight/obesity at 7 y had two to three times greater leptin values early in life (at 7 y), compared to their under/normal weight peers, representing a large effect size, with correlations of leptin and BMIz at both ages ranging between 0.56 and 0.70 ($p < 0.001$) among boys and girls respectively. No group differences were found in leptin levels from umbilical cord blood.

With the exception of rare genetic forms of leptin deficiency or mutations, circulating leptin correlates with BMI and total fat mass (Bates and Myers, 2003; Cohen et al., 2018; Olza et al., 2017; Wasim et al., 2016). Higher leptin among overweight individuals suggests possible leptin resistance and may be due to the disturbance of central and peripheral mechanisms (Olza et al., 2017). Importantly, leptin may be a biomarker of metabolic dysfunction among prepubertal children, with a 3% increased odds of metabolic syndrome for every 1-ng/dL increase of circulating leptin (Madeira et al., 2016). This leptin-associated cardiometabolic risk was also seen in a sample of European children, independent of child BMI and total fat mass (Nappo et al., 2017), and thereby potentially mediating or exacerbating the negative health consequences of obesity. Notably, even among children in the overweight/obese category, serum leptin is strongly correlated with the degree of adiposity (Miller et al., 2014; Olza et al., 2017).

We hypothesized that higher leptin levels at 7 years would be associated with higher scores on food approach behaviors. Even though leptin is an anorexigenic (appetite-reducing) hormone, leptin levels at 7 y were indeed cross-sectionally associated with higher food approach (Food Responsiveness, Enjoyment of Food, Emotional Overeating) at 7 y and 10 y, independent of potential confounders (Model 1). These findings are consistent with a model in which leptin resistance, reflected here by higher leptin levels, produces a weakened anorexigenic effect of leptin and thus promotes heightened food approach. Corroborating this interpretation, other studies of children and adolescents have shown associations between higher levels of leptin and

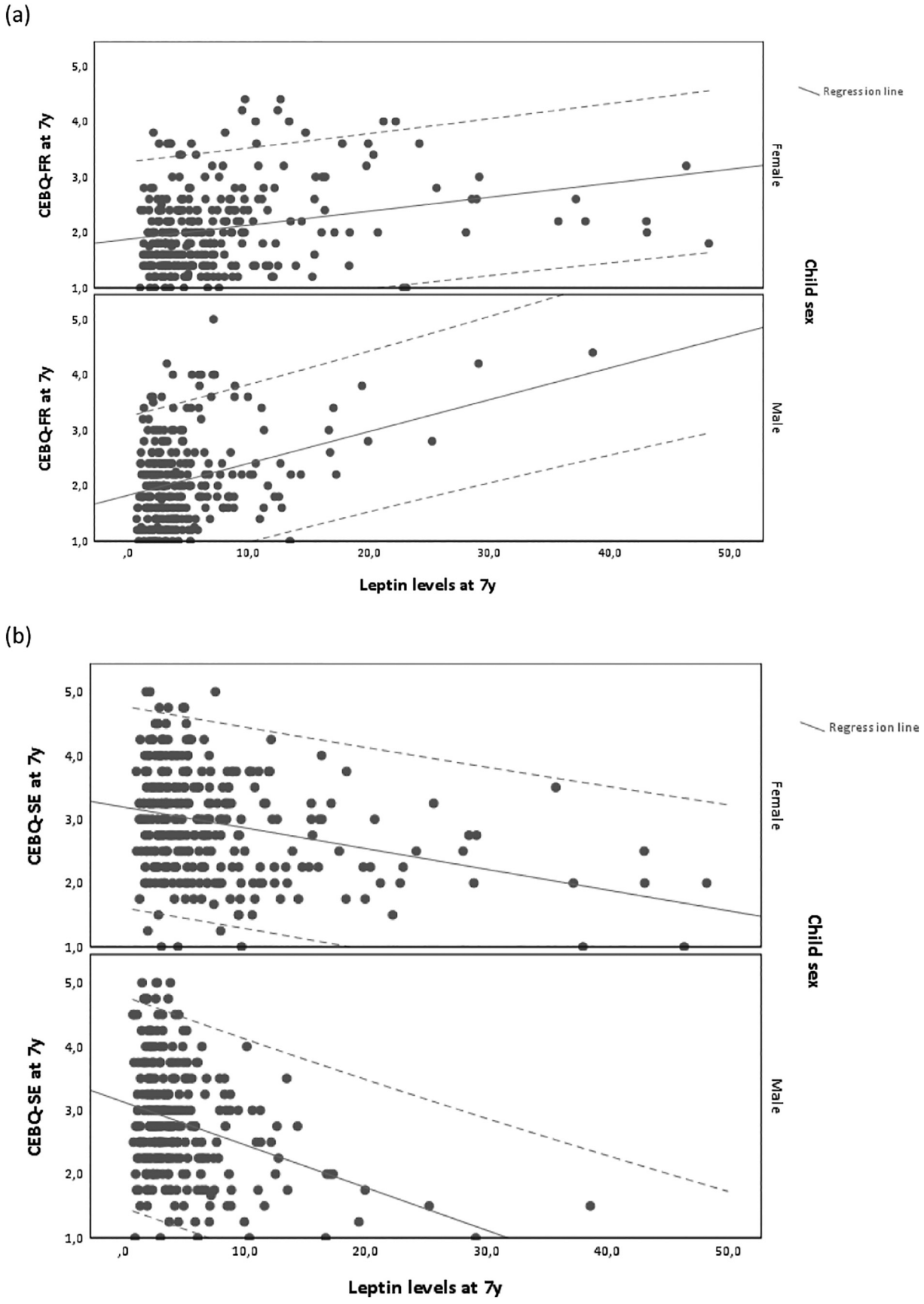

Fig. 1. Scatterplots illustrating associations between leptin levels (at 7 years old) and Food Responsiveness (CEBQ-FR) (a) and Slowness in Eating (CEBQ-SE) (b) at the same age for females and males.

Table 3
Generalized linear models assessing prospective relationships between leptin at 7 years old and appetitive behaviors at age 10.

	Food avoidant behaviors				Food approach behaviors			
	CEBQ-SR	CEBQ-SE	CEBQ-FF	CEBQ-EUE	CEBQ-FR	CEBQ-EF	CEBQ-DD	CEBQ-EOE
	β (95% CI)							
Model 1	**−0.01 (−0.01; 0.00)**	−0.01 (−0.01; 0.00)	0.00 (−0.01; 0.00)	0.00 (−0.01; 0.00)	**0.02 (0.01; 0.02)**	**0.01 (0.00; 0.02)**	0.00 (−0.01; 0.01)	**0.01 (0.00; 0.02)**
Model 2	0.00 (−0.01; 0.01)	0.00 (−0.01; 0.01)	0.00 (−0.01; 0.01)	0.00 (−0.01; 0.01)	0.00 (−0.01; 0.01)	0.00 (−0.01; 0.01)	0.00 (−0.01; 0.01)	0.00 (−0.01; 0.01)
Model 3	0.00 (−0.01; 0.01)	−0.01 (−0.03; 0.01)	0.00 (−0.01; 0.02)	0.01 (−0.01; 0.03)	0.00 (−0.01; 0.02)	0.00 (−0.01; 0.01)	−0.01 (−0.03; 0.00)	0.01 (−0.01; 0.02)
Leptin × BMIz interaction	0.00 (−0.01; 0.01)	0.01 (0.00; 0.02)	0.00 (−0.01; 0.01)	−0.01 (−0.02; 0.00)	0.00 (−0.01; 0.01)	0.00 (−0.01; 0.01)	0.01 (0.00; 0.01)	0.00 (−0.01; 0.00)
Model 4a	0.01 (0.00; 0.03)	0.01 (−0.01; 0.03)	0.01 (−0.01; 0.02)	0.00 (−0.02; 0.02)	−0.01 (−0.02; 0.01)	−0.01 (−0.02; 0.01)	−0.01 (−0.03; 0.01)	0.00 (−0.01; 0.02)
Leptin × Sex interaction	−0.01 (−0.03; 0.00)	−0.01 (−0.03; 0.01)	0.00 (−0.01; 0.02)	−0.01 (−0.03; 0.01)	0.00 (−0.02; 0.02)	0.00 (−0.02; 0.02)	0.01 (−0.01; 0.03)	0.00 (0.02; 0.01)
Model 4b	0.00 (−0.01; 0.01)	0.00 (−0.02; 0.02)	−0.01 (−0.02; 0.01)	0.00 (−0.02; 0.02)	**0.02 (0.00; 0.04)**	0.01 (0.00; 0.03)	0.00 (−0.02; 0.01)	**0.02 (0.00; 0.03)**
Leptin × Sex interaction	−0.01 (−0.03; 0.01)	−0.01 (−0.03; 0.01)	0.01 (−0.01; 0.02)	−0.01 (−0.03; 0.01)	0.00 (−0.02; 0.02)	0.00 (−0.02; 0.02)	0.01 (−0.01; 0.03)	−0.01 (−0.03; 0.01)

CI: confidence intervals; CEBQ: Children's Eating Behavior Questionnaire. CEBQ-SR: Satiety Responsiveness; CEBQ-SE: Slowness in Eating; CEBQ-FF: Food Fussiness; CEBQ-EUE: Emotional Undereating; CEBQ-FR: Food Responsiveness; CEBQ-EF: Enjoyment of Food; CEBQ-DD: Desire to Drink; CEBQ-EOE: Emotional Overeating. Scores range from 1 - 'never' to 5 - 'always'. Model 1 adjusted for maternal education, maternal age, pre-pregnancy BMI, smoking during pregnancy, child mean daily time of physical activity at 7 y and the respective eating behavior at 7 y. Model 2 adjusted for Model 1 plus child BMIz at 10 y. Model 3 adjusted for Model 2 plus interaction term Leptin levels at 7 y × BMIz. Model 4a adjusted for Model 2 plus interaction term Leptin levels at 7 y × child sex (female as reference category). Model 4b adjusted for Model 1 plus interaction term Leptin levels at 7 y × child sex (female as reference category). **Bold** indicates significant main effect of leptin levels or interaction terms ($p < 0.05$).

greater eating in absence of hunger and greater energy consumption in a standardized behavioral test (Fisher et al., 2007), and self-reported loss of control over eating (Miller et al., 2014).

Divergent to what was previously found in a Canadian sample of school-aged children (Cohen et al., 2018), we did not see a relationship between leptin levels and Desire to Drink. A limitation of the Desire to Drink sub-scale is that it does not describe the type of beverage the child has motivation to consume and therefore does not specifically assess consumption of sugary drinks – a well-known eating behavior linked to childhood obesity (Keller and Bucher Della Torre, 2015). Our failure to see a relationship between leptin and Desire to Drink could therefore be attributable to differing eating patterns and interpretations of question items between countries. However, it is also possible that leptin has stronger, and therefore more consistently observed, effects on approach of foods than drinks.

The majority of the associations we observed between leptin levels and food approach traits at 7 y did not remain significant when adjusting for child BMIz (Model 2). This suggests that many of these leptin-appetite associations may be reflective of known associations between body weight and appetite. However, it is also consistent with leptin affecting eating behaviors with downstream effects on body weight. Further, eating more in response to emotional stimuli (Emotional Overeating) was associated with greater leptin independently of child BMIz (Model 2). This finding builds upon results from a Chilean (Valladares et al., 2015) and Belgian (Michels et al., 2017) study, the latter of which showed that higher leptin levels were associated with greater effects of stress, measured by both a questionnaire on children's emotions and salivary cortisol measurements, and on emotional eating, measured by an eating behavior questionnaire, among girls (Michels et al., 2017). Consistent with both our findings and these other studies, leptin polymorphisms have been previously associated with greater preference for sweet taste, operationalized by a single questionnaire item assessing sweet taste preference among Japanese adults (Mizuta et al., 2008), and emotional eating primarily involves consumption of energy-dense comfort foods which are often high in sugar (Aparicio et al., 2017). The associations we observed may therefore be attributable to the action of leptin on dopaminergic circuits subserving reward and motivation (DiLeone, 2009).

To explore the possibility that the effect of leptin and appetite would differ depending on current levels of body fat, with heavier individuals showing more leptin resistance and therefore more obesogenic appetite profiles, we tested for interactions between leptin and BMIz. However, no interactions were observed in either the cross-sectional or the prospective analyses. This absence of effect may have been due to a lack of variance at the upper end of the weight distribution or the high correlation between child BMIz and leptin levels. Additional research, with larger sample sizes, is necessary to further understand a possible leptin resistance among children in higher weight categories and its effect of eating behaviors.

Due to known sex differences in body fat, leptin and appetite, we also explored whether leptin-appetite associations differed by sex. Consistent with previous findings (Christen et al., 2018; Jois et al., 2015), we observed greater leptin levels among girls. However, associations between leptin levels and appetitive traits were greater among boys (Model 4b). Specifically, for both sexes, higher leptin was associated with greater Food Responsiveness, with greater effects apparent among boys, potentially increasing the risk of future weight gain. As expected, these short-term effects (at 7 y) were more consistent than those observed three years later. Notably the results of Valladares et al. (2015) also varied by sex (Valladares et al., 2015). The sex difference we observed may be attributable to hormones, gender-specific genes or girls' higher body fat percentage (Christen et al., 2018; Jois et al., 2015) and pubertal development (Rogol, 2009), which are stimulating factors for leptin and leptin resistance.

Replication is necessary to confirm these exploratory findings. However, they are important as they extend the findings of previous studies exploring unadjusted associations between leptin and CEBQ scores (Cohen et al., 2018; Valladares et al., 2015), and suggest that for

some eating behavior relationships (Emotional Overeating) leptin levels are not simply a proxy for body weight, thereby recapitulating established associations between CEBQ sub-scales and body weight. Rather, leptin levels could be showing independent relationships with appetite.

Our second hypothesis was that leptin levels would be negatively associated with scores on food avoidant sub-scales, i.e. Satiety Responsiveness, Slowness in Eating, Food Fussiness and Emotional Undereating. Our results showed that leptin levels were indeed associated with lower Slowness in Eating scores at 7 years. Further, this effect was modified by sex, such that associations were stronger in boys (Model 4b). This is in contradiction to the Chilean study of children with obesity (Valladares et al., 2015), which found that girls with excessive weight and the presence of a leptin variant showed *higher* scores of Slowness in Eating. Our results suggest that, to a greater degree among boys, raised leptin levels failed to promote satiety. An inverse relation between leptin levels and satiety was also observed in a dietary intervention among obese 6–12 year-olds following a 2-month period consuming diets with low carbohydrate and low fat (Ibarra-Reynoso et al., 2015). Together these results support a model in which appetite and satiety result from the interplay of factors including leptin and leptin resistance, although further longitudinal studies are necessary to establish bidirectional associations between appetite and leptin levels over time.

Our last hypothesis was that umbilical cord blood leptin would predict appetitive behaviors in childhood. The evidence for long-term effects of in utero leptin on child health are variable in extant literature, with longitudinal studies showing negative (Boeke et al., 2013; Karakosta et al., 2016) and also null associations (Meyer et al., 2018) between cord leptin and later child weight. Cohort studies have demonstrated the association between cord blood leptin and greater maternal pre-pregnancy BMI and child birth weight (Karakosta et al., 2013). However the relationship between cord blood leptin and weight during childhood is unclear, with some studies showing no effects by preschool-age (Chaoimh et al., 2016; Mantzoros et al., 2009) and another showing associations with fat mass, waist circumference and BMIz at school-age, but not adolescence (Simpson et al., 2017). In the current study, umbilical cord blood leptin was not significantly different between children overweight/obese at 10 y and their non-overweight peers, and no prospective association was found with appetitive behaviors at age 10.

Our study had some limitations. Child eating behaviors were assessed using parent-report, which may introduce measurement error due to subjectivity and social desirability bias. However, CEBQ subscales have demonstrated good internal reliability in this population (Albuquerque et al., 2017) and good correspondence with objective behavioral measures of eating (Carnell and Wardle, 2007). BMIz is a good population measure of excessive weight in children but is an indirect measure of fat mass (Freedman et al., 2017; Frühbeck et al., 2019). Large longitudinal studies including frequent and contemporaneous assessments of eating behaviors, leptin levels and adiposity throughout development are necessary to confirm these findings and inform on causal relationships. Despite these limitations, this is one of the first studies to investigate prospective as well as cross-sectional associations between leptin levels and specific appetitive behaviors during the school-age years. Strengths include the population-based prospective design and the relatively large number of available leptin samples at birth and at 7 years.

5. Conclusions

To summarize, leptin levels were positively associated with food approach and negatively with food avoidant behaviors. Associations tended to be stronger in boys than girls, and were more consistent in cross-sectional analyses using data from children at 7 y of age. Given the correlation between leptin levels and body weight it is challenging to demonstrate relationships between leptin and appetitive behaviors that are independent of body weight. Nonetheless, we here observed some associations between leptin levels and appetitive traits, even when controlling for child BMIz at each age; this supports a potential role for leptin in affecting appetite, with consequences for both current weight status, and potential future weight gain. Future longitudinal research with larger samples of overweight and obese children could help to clarify our findings and conclusions. However, our results suggest that leptin resistance, which promotes overeating and mitigates against weight loss in adulthood, may occur with measurable impacts on eating behavior in early childhood. Interventions targeting appetitive behaviors to prevent obesity may therefore be beneficial during relatively early stages of eating behavior development. The long-term effects of leptin resistance on children's eating behaviors and obesity risk merit further study.

Declaration of competing interest

None.

Acknowledgements

The authors gratefully acknowledge the families enrolled in Generation XXI for their kindness, all members of the research team for their enthusiasm and perseverance and the participating hospitals and their staff for their help and support. We also acknowledge the support from the Epidemiology Research Unit (EPI-Unit: UIDB/04750/2020; POCI-01-0145-FEDER-006862).

Funding

Generation XXI was funded by the Health Operational Programme – Saúde XXI, Community Support Framework III and the Regional Department of Ministry of Health. This study was supported through FEDER from the Operational Programme Factors of Competitiveness – COMPETE and through national funding from the Foundation for Science and Technology – FCT (Portuguese Ministry of Education and Science) under the projects “Appetite regulation and obesity in childhood: a comprehensive approach towards understanding genetic and behavioural influences” (POCI-01-0145-FEDER-030334; PTDC/SAU-EPI/30334/2017); “Appetite and adiposity - evidence for gene-environment interplay in children” (IF/01350/2015); “HIneC: When do health inequalities start? Understanding the impact of childhood social adversity on health trajectories from birth to early adolescence” (POCI-01-0145-FEDER-029567; Reference: PTDC/SAU-PUB/29567/2017) and through Investigator Contract (IF/01350/2015 - Andreia Oliveira). It had also support from the Calouste Gulbenkian Foundation, Portugal.

References

Aiken, L., West, S., 1991. Multiple Regression: Testing and Interpreting Interactions. Sage, Newbury Park, CA.

Albuquerque, G., Severo, M., Oliveira, A., 2017. Early life characteristics associated with appetite-related eating behaviors in 7-year-old children. J. Pediatr. 180, 38–46.e2. https://doi.org/10.1016/j.jpeds.2016.09.011.

Alves, E., Correia, S., Barros, H., Azevedo, A., 2012. Prevalence of self-reported cardiovascular risk factors in Portuguese women: a survey after delivery. Int. J. Public Health 57, 837–847. https://doi.org/10.1007/s00038-012-0340-6.

Aparicio, E., Canals, J., Voltas, N., Valenzano, A., Arija, V., 2017. Emotional symptoms and dietary patterns in early adolescence: a school-based follow-up study. J. Nutr. Educ. Behav. 49, 405–414.e1. https://doi.org/10.1016/j.jneb.2017.01.015.

Bates, S.H., Myers, M.G., 2003. The role of leptin receptor signaling in feeding and neuroendocrine function. Trends Endocrinol. Metab. 14, 447–452. https://doi.org/10.1016/j.tem.2003.10.003.

Berger, P.K., Hohman, E.E., Marini, M.E., Savage, J.S., Birch, L.L., 2016. Girls' picky eating in childhood is associated with normal weight status from ages 5 to 15 y. Am. J. Clin. Nutr. 104, 1577–1582. https://doi.org/10.3945/ajcn.116.142430.

Boeke, C.E., Mantzoros, C.S., Hughes, M.D., Rifas-Shiman, S.L., Villamor, E., Zera, C.A., Gillman, M.W., 2013. Differential associations of leptin with adiposity across early childhood. Obesity 21, 1430–1437. https://doi.org/10.1002/oby.20314.

Cao, H., 2014. Adipocytokines in obesity and metabolic disease. J. Endocrinol. 220, T47–T59. https://doi.org/10.1530/joe-13-0339.

Carnell, S., Wardle, J., 2007. Measuring behavioural susceptibility to obesity: validation of the child eating behaviour questionnaire. Appetite 48, 104–113. https://doi.org/10.1016/j.appet.2006.07.075.

Chaoimh, C., Murray, D.M., Kenny, L.C., Irvine, A.D., O'Hourihane, J.B., Kiely, M., 2016. Cord blood leptin and gains in body weight and fat mass during infancy. Eur. J. Endocrinol. 175, 403–410. https://doi.org/10.1530/EJE-16-0431.

Christen, T., Trompet, S., Noordam, R., van Klinken, J.B., van Dijk, K.W., Lamb, H.J., Cobbaert, C.M., den Heijer, M., Jazet, I.M., Jukema, J.W., Rosendaal, F.R., de Mutsert, R., 2018. Sex differences in body fat distribution are related to sex differences in serum leptin and adiponectin. Peptides 107, 25–31. https://doi.org/10.1016/j.peptides.2018.07.008.

Cohen, J., 1988. Statistical Power Analysis for the Behavioral Sciences, 2nd ed. Lawrence Erlbaum Associates, New York.

Cohen, J., Cohen, P., West, S., Aiken, L., 2003. Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences, 3rd ed. Routledge, New York.

Cohen, T.R., Hazell, T.J., Vanstone, C.A., Rodd, C., Weiler, H.A., 2018. Changes in eating behavior and plasma leptin in children with obesity participating in a family-centered lifestyle intervention. Appetite 125, 81–89. https://doi.org/10.1016/j.appet.2018.01.017.

Costa, A, Severo, M, Vilela, S, Fildes, A, Oliveira, A, 2020. Bidirectional relationships between appetitive behaviours and body mass index in childhood: a cross-lagged analysis in the Generation XXI birth cohort. Eur J Nutr. https://doi.org/10.1007/s00394-020-02238-9. In press.

de Lauzon, B., Romon, M., Deschamps, V., Lafay, L., Borys, J.-M., Karlsson, J., Ducimetière, P., Charles, M.A., Fleurbaix Laventie Ville Sante Study Group, 2004. The three-factor eating questionnaire-R18 is able to distinguish among different eating patterns in a general population. J. Nutr. 134, 2372–2380. https://doi.org/10.1093/jn/134.9.2372.

de Onis, M., Onyango, A.W., Borghi, E., Siyam, A., Nishida, C., Siekmann, J., 2007. Development of a WHO growth reference for school-aged children and adolescents. Bull. World Health Organ. 85, 660–667.

Derks, I.P.M., Sijbrands, E.J.G., Wake, M., Qureshi, F., van der Ende, J., Hillegers, M.H.J., Jaddoe, V.W.V., Tiemeier, H., Jansen, P.W., 2018. Eating behavior and body composition across childhood: a prospective cohort study. Int. J. Behav. Nutr. Phys. Act. 15, 96. https://doi.org/10.1186/s12966-018-0725-x.

DiLeone, R.J., 2009. The influence of leptin on the dopamine system and implications for ingestive behavior. Int. J. Obes. 33, S25–S29. https://doi.org/10.1038/ijo.2009.68.

Farr, O.M., Tsoukas, M.A., Mantzoros, C.S., 2015. Leptin and the brain: influences on brain development, cognitive functioning and psychiatric disorders. Metabolism 64, 114–130. https://doi.org/10.1016/j.metabol.2014.07.004.

Fisher, J.O., Cai, G., Jaramillo, S.J., Cole, S.A., Comuzzie, A.G., Butte, N.F., 2007. Heritability of hyperphagic eating behavior and appetite-related hormones among hispanic children. Obesity 15, 1484–1495. https://doi.org/10.1038/oby.2007.177.

Freedman, D.S., Butte, N.F., Taveras, E.M., Lundeen, E.A., Blanck, H.M., Goodman, A.B., Ogden, C.L., 2017. BMI z-Scores are a poor indicator of adiposity among 2- to 19-year-olds with very high BMIs, NHANES 1999–2000 to 2013–2014. Obesity 25, 739–746. https://doi.org/10.1002/oby.21782.

Frühbeck, G., Busetto, L., Dicker, D., Yumuk, V., Goossens, G.H., Hebebrand, J., Halford, J.G.C., Farpour-Lambert, N.J., Blaak, E.E., Woodward, E., Toplak, H., 2019. The ABCD of obesity: an EASO position statement on a diagnostic term with clinical and scientific implications. Obes. Facts 131–136. https://doi.org/10.1159/000497124.

Galic, S., Oakhill, J.S., Steinberg, G.R., 2010. Adipose tissue as an endocrine organ. Mol. Cell. Endocrinol. 316, 129–139. https://doi.org/10.1016/j.mce.2009.08.018.

Gil-Campos, M., Aguilera, C.M., Ramirez-Tortosa, M.C., Cañete, R., Gil, A., 2010. Fasting and postprandial relationships among plasma leptin, ghrelin, and insulin in prepubertal obese children. Clin. Nutr. 29, 54–59. https://doi.org/10.1016/j.clnu.2009.06.007.

Husted, J.A., Cook, R.J., Farewell, V.T., Gladman, D.D., 2000. Methods for assessing responsiveness: a critical review and recommendations. J. Clin. Epidemiol. 53, 459–468. https://doi.org/10.1016/S0895-4356(99)00206-1.

Ibarra-Reynoso, L.D.R., Pisarchyk, L., Pérez-Luque, E.L., Garay-Sevilla, M.E., Malacara, J.M., 2015. Dietary restriction in obese children and its relation with eating behavior, fibroblast growth factor 21 and leptin: a prospective clinical intervention study. Nutr. Metab. 12, 31. https://doi.org/10.1186/s12986-015-0027-0.

Jois, A., Navarro, P., Ortega-Senovilla, H., Gavela-Pérez, T., Soriano-Guillén, L., Garcés, C., 2015. Relationship of high leptin levels with an adverse lipid and insulin profile in 6-8 year-old children in Spain. Nutr. Metab. Cardiovasc. Dis. 25, 1111–1116. https://doi.org/10.1016/j.numecd.2015.09.005.

Kanoski, S.E., Hayes, M.R., Greenwald, H.S., Fortin, S.M., Gianessi, C.A., Gilbert, J.R., Grill, H.J., 2011. Hippocampal leptin signaling reduces food intake and modulates food-related memory processing. Neuropsychopharmacology 36, 1859–1870. https://doi.org/10.1038/npp.2011.70.

Karakosta, P., Georgiou, V., Fthenou, E., Papadopoulou, E., Roumeliotaki, T., Margioris, A., Castanas, E., Kampa, M., Kogevinas, M., Chatzi, L., 2013. Maternal weight status, cord blood leptin and fetal growth: a prospective mother-child cohort study (Rhea study). Paediatr. Perinat. Epidemiol. 27, 461–471. https://doi.org/10.1111/ppe.12074.

Karakosta, P., Roumeliotaki, T., Chalkiadaki, G., Sarri, K., Vassilaki, M., Venihaki, M., Malliaraki, N., Kampa, M., Castanas, E., Kogevinas, M., Mantzoros, C., Chatzi, L., 2016. Cord blood leptin levels in relation to child growth trajectories. Metabolism 65, 874–882. https://doi.org/10.1016/j.metabol.2016.03.003.

Keller, A., Bucher Della Torre, S., 2015. Sugar-sweetened beverages and obesity among children and adolescents: a review of systematic literature reviews. Child. Obes. 11, 338–346. https://doi.org/10.1089/chi.2014.0117.

Kolko, R.P., Salk, R.H., Sweeny, G.M., Marcus, M.D., Levine, M.D., 2018. Mothers' loss of control over eating during pregnancy in relation to their infants' appetitive traits. Appetite 120, 1–5. https://doi.org/10.1016/j.appet.2017.08.007.

Larsen, P.S., Kamper-Jørgensen, M., Adamson, A., Barros, H., Bonde, J.P., Brescianini, S., Brophy, S., Casas, M., Devereux, G., Eggesbø, M., Fantini, M.P., Frey, U., Gehring, U., Grazuleviciene, R., Henriksen, T.B., Hertz-Picciotto, I., Heude, B., Hryhorczuk, D.O., Inskip, H., Jaddoe, V.W.V., Lawlor, D.A., Ludvigsson, J., Kelleher, C., Kiess, W., Koletzko, B., Kuehni, C.E., Kull, I., Kyhl, H.B., Magnus, P., Momas, I., Murray, D., Pekkanen, J., Polanska, K., Porta, D., Poulsen, G., Richiardi, L., Roeleveld, N., Skovgaard, A.M., Sram, R.J., Strandberg-Larsen, K., Thijs, C., Van Eijsden, M., Wright, J., Vrijheid, M., Andersen, A.-M.N., 2013. Pregnancy and birth cohort resources in Europe: a large opportunity for aetiological child health research. Paediatr. Perinat. Epidemiol. 27, 393–414. https://doi.org/10.1111/ppe.12060.

Lenhard, W., Lenhard, A., 2016. Calculation of Effect Sizes. Psychometrica, Dettelbach (Germany). https://doi.org/10.13140/RG.2.1.3478.4245. http://www.psychometrica.de/effect_size.html, Accessed date: 23 April 2020.

Madeira, I., Bordallo, M.A., Rodrigues, N.C., Carvalho, C., Gazolla, F., Collett-Solberg, P., Medeiros, C., Bordallo, A.P., Borges, M., Monteiro, C., Ribeiro, R., 2016. Leptin as a predictor of metabolic syndrome in prepubertal children. Arch. Endocrinol. Metab. 61, 7–13. https://doi.org/10.1590/2359-3997000000199.

Mantzoros, C.S., Rifas-Shiman, S.L., Williams, C.J., Fargnoli, J.L., Kelesidis, T., Gillman, M.W., 2009. Cord blood leptin and adiponectin as predictors of adiposity in children at 3 years of age: a prospective cohort study. Pediatrics 123, 682–689. https://doi.org/10.1542/peds.2008-0343.

Meyer, D.M., Brei, C., Stecher, L., Much, D., Brunner, S., Hauner, H., 2018. Leptin in maternal plasma and cord blood as a predictor of offspring adiposity at 5 years: a follow-up study. Obesity 26, 279–283. https://doi.org/10.1002/oby.22037.

Mi, J., Munkonda, M.N., Li, M., Zhang, M.-X., Zhao, X.-Y., Fouejeu, P.C.W., Cianflone, K., 2010. Adiponectin and leptin metabolic biomarkers in Chinese children and adolescents. J. Obes. 2010, 892081. https://doi.org/10.1155/2010/892081.

Michels, N., Sioen, I., Ruige, J., De Henauw, S., 2017. Children's psychosocial stress and emotional eating: a role for leptin? Int. J. Eat. Disord. 50, 471–480. https://doi.org/10.1002/eat.22593.

Miller, R., Tanofsky-Kraff, M., Shomaker, L.B., Field, S.E., Hannallah, L., Reina, S.A., Mooreville, M., Sedaka, N., Brady, S.M., Condarco, T., Reynolds, J.C., Yanovski, S.Z., Yanovski, J.A., 2014. Serum leptin and loss of control eating in children and adolescents. Int. J. Obes. 38, 397–403. https://doi.org/10.1038/ijo.2013.126.

Mizuta, E., Kokubo, Y., Yamanaka, I., Miyamoto, Y., Okayama, A., Yoshimasa, Y., Tomoike, H., Morisaki, H., Morisaki, T., 2008. Leptin gene and leptin receptor gene polymorphisms are associated with sweet preference and obesity. Hypertens. Res. 31, 1069–1077. https://doi.org/10.1291/hypres.31.1069.

Murray, S., Tulloch, A., Gold, M.S., Avena, N.M., 2014. Hormonal and neural mechanisms of food reward, eating behaviour and obesity. Nat. Rev. Endocrinol. 10, 540–552. https://doi.org/10.1038/nrendo.2014.91.

Nappo, A., González-Gil, E.M., Ahrens, W., Bammann, K., Michels, N., Moreno, L.A., Kourides, Y., Iacoviello, L., Mårild, S., Fraterman, A., Molnàr, D., Veidebaum, T., Siani, A., Russo, P., 2017. Analysis of the association of leptin and adiponectin concentrations with metabolic syndrome in children: results from the IDEFICS study. Nutr. Metab. Cardiovasc. Dis. 27, 543–551. https://doi.org/10.1016/j.numecd.2017.04.003.

Olza, J., Rupérez, A.I., Gil-Campos, M., Leis, R., Cañete, R., Tojo, R., Gil, Á., Aguilera, C.M., 2017. Leptin receptor gene variant rs11804091 is associated with BMI and insulin resistance in Spanish female obese children: a case-control study. Int. J. Mol. Sci. 18, 1690. https://doi.org/10.3390/ijms18081690.

Plonka, M., Toton-Morys, A., Adamski, P., Bielanski, W., Dobrzanska, M.J., Kaminska, A., Piorecka, B., Glodzik, J., 2011. Association of the physical activity with leptin blood serum level, body mass indices and obesity in schoolgirls. J. Physiol. Pharmacol. 62, 647–656.

Rahill, S., Kennedy, A., Walton, J., McNulty, B.A., Kearney, J., 2019. The factors associated with food fussiness in Irish school-aged children. Public Health Nutr. 22, 164–174. https://doi.org/10.1017/S1368980018002835.

Rogol, A.D., 2009. Sex steroids, growth hormone, leptin and the pubertal growth spurt. Endocr. Dev. 17, 77–85. https://doi.org/10.1159/000262530.

Rückinger, S., Beyerlein, A., Jacobsen, G., von Kries, R., Vik, T., 2010. Growth in utero and body mass index at age 5years in children of smoking and non-smoking mothers. Early Hum. Dev. 86, 773–777. https://doi.org/10.1016/j.earlhumdev.2010.08.027.

Schrempft, S., van Jaarsveld, C.H.M., Fisher, A., Fildes, A., Wardle, J., 2016. Maternal characteristics associated with the obesogenic quality of the home environment in early childhood. Appetite 107, 392–397. https://doi.org/10.1016/j.appet.2016.08.108.

Simpson, J., Smith, A.D.A.C., Fraser, A., Sattar, N., Lindsay, R.S., Ring, S.M., Tilling, K., Smith, G.D., Lawlor, D.A., Nelson, S.M., 2017. Programming of adiposity in childhood and adolescence: associations with birth weight and cord blood adipokines. J. Clin. Endocrinol. Metab. 102, 499–506. https://doi.org/10.1210/jc.2016-2342.

Valladares, M., Obregón, A.M., Weisstaub, G., Burrows, R., Patiño, A., Ho-Urriola, J., Santos, J.L., 2015. Asociación entre la conducta alimentaria y polimorfismos genéticos de la leptina y su receptor en niños obesos chilenos. Nutr. Hosp. 31, 1044–1051. https://doi.org/10.3305/nh.2015.31.3.8049.

van Jaarsveld, C.H.M., Llewellyn, C.H., Johnson, L., Wardle, J., 2011. Prospective associations between appetitive traits and weight gain in infancy. Am. J. Clin. Nutr. 94, 1562–1567. https://doi.org/10.3945/ajcn.111.015818.Individual.

Wardle, J., Guthrie, C.A., Sanderson, S., Rapoport, L., 2001. Development of the Children's Eating Behaviour Questionnaire. J. Child Psychol. Psychiatry 42, 963–970. https://doi.org/10.1111/1469-7610.00792.

Wasim, M., Awan, F.R., Najam, S.S., Khan, A.R., Khan, H.N., 2016. Role of leptin deficiency, inefficiency, and leptin receptors in obesity. Biochem. Genet. 54, 565–572. https://doi.org/10.1007/s10528-016-9751-z.

Webber, L., Hill, C., Saxton, J., van Jaarsveld, C.H.M., Wardle, J., 2009. Eating behaviour and weight in children. Int. J. Obes. 33, 21–28. https://doi.org/10.1038/ijo.2008.219.

World Health Organization (WHO), 2018. Facts Sheet: Obesity and Overweight.