

Sexual Dimorphism in the Early Life Programming of Serum Leptin Levels in European Adolescents: The HELENA Study

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Aim: The aim of this study was to test the hypothesis that a lower birth weight, as an indicator of adverse intrauterine environment, is associated with higher serum leptin levels in European adolescents. We also examined the possible sexual dimorphism in this relationship.

Methods: Fasting serum leptin was measured in 757 European born at term adolescents (429 females) aged 14.6 ± 1.2 yr. We measured weight and height, and body mass index was calculated. Birth weight, duration of pregnancy, and duration of breast-feeding were obtained from parental records. Duration of pregnancy and breast-feeding, pubertal status, center, body mass index, and physical activity were entered as confounders in the analyses.

Results: There was a significant interaction effect between sex and birth weight on serum leptin levels ($P = 0.044$). We observed that body weight at birth was negatively and significantly associated with serum leptin levels only in female adolescents ($\beta = -0.109$; adjusted $P = 0.008$). The association persisted after further controlling for physical activity ($\beta = -0.115$; adjusted $P = 0.016$).

Conclusions: These findings provide further evidence for a sex-specific programming effect of birth weight on serum leptin levels. Our results also contribute to explain the detrimental health effects associated with lower birth weight, such as long-term increased risk of developing obesity and type 2 diabetes. (*J Clin Endocrinol Metab* 96: E1330–E1334, 2011)

There is increasing evidence indicating that people exposed to metabolic and nutritional alteration during fetal environment may have increased risk of developing obesity and type 2 diabetes in adulthood (1). It has been suggested that the concentration of hormones, metabolites, and neurotransmitters during critical periods of early development can preprogram brain development and metabolism later in life, yet the mechanisms are unclear (2). In addition, previous studies have identified marked sex differences in programming effects (3).

Leptin is a hormone mainly produced and secreted into the circulation by the adipose tissue and plays a key role in the chronic control of energy balance and insulin sensitivity. It informs the hypothalamus and other central areas about nutritional status and fat stores. Leptin increases energy expenditure and exerts an anorectic effect on appetite. In obesity, there is a sustained high leptin circulating level and leptin resistance status at the hypothalamic level. Accordingly, leptin is a strong candidate for playing a key role in the mechanism involved in the fetal hypothalamic programming of energy homeostasis and hence in development of obesity and type 2 diabetes (4).

In this study we tested the hypothesis that a lower birth weight, as an indicator of adverse intrauterine environment, may be associated with higher serum leptin levels in European adolescents. We also examined the possible sexual dimorphism in this relationship.

Materials and Methods

Study design and sample

Participants in the current study belonged from the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) cross-sectional study. This epidemiological study examines environmental and lifestyle factors in European adolescents as well as their influence on future cardiovascular disease risks (5). Details of the study design, selection criteria, sample calculations, standardization and harmonization processes, and quality control activities can be found elsewhere (6, 7). The Human Research Review Committee of the universities of the centers involved approved the protocol. Parents and adolescent provided written informed consent to participate in the study.

The present study comprised a total of 757 adolescents born at term (>37 wk of amenorrhea) with complete and valid data on birth weight, serum leptin concentration, body mass index (BMI), and pubertal status. There were no differences in the study key characteristics (*i.e.* age or BMI) between the present sample and the original HELENA sample ($n = 3546$, all $P > 0.1$).

Measurements

Body weight at birth, duration of gestation, and duration of breast-feeding (in months) were collected by parental records in a questionnaire. Parents were asked to recall this information from the health booklets of their son/daughter (8). The duration

of gestation was reported in two categories: between 37 and 40 wk and more than 40 wk of gestation.

We measured body weight and height, waist circumference, and subscapular and tricipital skinfold thicknesses in triplicate, and the mean was used in all analyses (9). BMI was calculated by dividing body weight (kilograms) with the square of height (meters). The SD score of BMI (Z-score BMI) was calculated using the *lmsGrowth* method (10). Body fat percentage was estimated using the equations reported by Slaughter *et al.* (11), as suggested elsewhere (12).

Sexual maturation status was assessed by a trained physician according to Tanner and Whitehouse (13). We measured physical activity with accelerometry over 7 d (Actigraph, GT1M, Pensacola, FL) (14) and was expressed as total counts per minute.

We measured serum fasting leptin concentrations in duplicate using the RayBio human leptin ELISA (Norcross, GA). The sensitivity of leptin assay was typically less than 6 pg/ml, with intra- and interassay coefficients of variation of less than 10% and less than 12%.

Statistical analysis

Characteristics of the study sample by sex are presented as means and SD, unless otherwise stated. Serum leptin presented a skewed distribution and was therefore logarithmically transformed. Analyses were performed using the SPSS, version 17.0 (SPSS Inc., Chicago, IL), and the level of significance was set to $P = 0.05$.

Regression analysis was used to examine the association between birth weight and serum leptin levels adjusting for duration of pregnancy, duration of breastfeeding, pubertal status, center (entered as dummy variable), and BMI. All the analyses were repeated controlling for Z-score BMI, body fat percentage, and waist circumference and height instead of BMI.

Results

Characteristics of the study sample are shown in Table 1.

There was a significant interaction effect between birth weight and sex on serum leptin levels ($P = 0.04$). Therefore, the analyses were stratified by sex. We observed that body weight at birth was negatively and significantly associated with serum leptin levels in female adolescents regardless of center, duration of gestation and breast-feeding, pubertal status, and BMI (Fig. 1). Further controlling for physical activity did not change the results ($\beta = -0.115$; $P = 0.016$). Likewise, the results did not substantially change when the analysis was controlled for Z-score BMI (-0.119 ; $P = 0.003$), body fat percentage ($\beta = -0.100$, $P = 0.015$), or waist circumference ($\beta = -0.117$, $P = 0.006$) instead of BMI.

We did not find any significant association between birth weight and serum leptin levels in male adolescents (Fig. 1). These results did not differ when the analyses were restricted to male at late puberty ($\beta = 0.001$, $P = 0.979$), *i.e.* excluding those males at Tanner stages I and II ($n = 37$) or when the analysis was controlled for Z-score BMI ($\beta =$

TABLE 1. Characteristics of adolescents

	Males (n = 328)	Females (n = 429)
Age (yr)	14.7 (1.3)	14.8 (1.2)
Body weight at birth (kg)	3.4 (0.6)	3.3 (0.5)
Duration of gestation (%)		
37–40 wk	70.2	74.7
≥40 wk	29.8	25.3
Breast-feeding duration (months)	3.0 (5.1)	4.2 (4.8)
Sexual maturation (%)		
Tanner stage I	1.4	
Tanner stage II	11.5	4.8
Tanner stage III	20.3	21.2
Tanner stage IV	35.7	44.4
Tanner stage V	31.1	29.5
BMI (kg/m ²)	20.9 (3.7)	21.2 (3.5)
Body fat percentage	19.2 (10.4)	26.0 (7.3)
Waist circumference (cm)	73.3 (8.8)	70.3 (7.9)
Nonobese overweight (n, %)	56, 17.1	65, 15.2
Obese (n, %)	21, 6.4	18, 4.2
Leptin (ng/ml)	9.4 (14.9)	28.4 (25.6)
Physical activity (counts/min) ^a	491 (154)	387 (124)

Data are means (SD).

^a Valid data on 502 adolescents (209 males and 293 females).

0.025, $P = 0.580$), body fat percentage ($\beta = -0.015$, $P = 0.728$), or waist circumference ($\beta = -0.046$, $P = 0.336$) instead of BMI.

The outcome did not substantially differ when obese ($\beta = -0.1198$, $P = 0.009$ and $\beta = -0.055$, $P = 0.320$ for females and males, respectively) or overweight adolescents ($\beta = -0.120$, $P = 0.019$ and $\beta = -0.024$, $P = 0.693$ for females and males, respectively) were excluded from the analysis.

Discussion

The present study shows a negative relationship between birth weight and serum leptin levels in female adolescents. This relationship was independent of several confounders

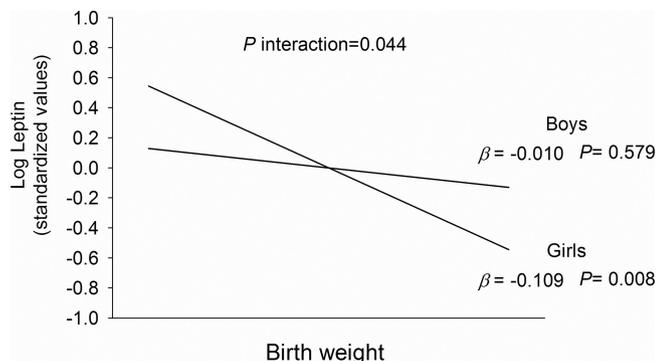


FIG. 1. Association between birth weight (log transformed) and serum leptin levels by sex in adolescents from the HELENA study. The models were adjusted for duration of pregnancy, duration of breast-feeding, pubertal status, center, and body mass index.

including total or central adiposity content, breast-feeding duration, pubertal status, duration of gestation, and physical activity. Likewise, the association between birth weight and serum leptin concentrations, regardless of adiposity, persisted after the exclusion of overweight adolescents from the analysis. To our knowledge, this is the first study reporting this association in adolescents and taking into account confounders affecting either birth weight, leptin, or adiposity estimates.

Leptin is the major peripheral hormone involved in long-term energy homeostasis. In obesity, the existence of an endogenous leptin-resistance mechanism limiting its regulatory effect may explain the strong correlation between serum leptin concentrations and body fat mass (15). In addition, there is evidence that leptin plays a role during the neonatal development in key areas of the hypothalamus that are involved in the central regulation of energy balance (2).

The role of leptin in the fetal programming of later obesity and type 2 diabetes mainly comes from research in animal models (16). In rodents, previous studies showed that alterations in leptin concentrations during the key perinatal periods of hypothalamic development may program selective leptin resistance (17). Neonatal leptin is required to the adequate development of hypothalamic arcuate nucleus and later functioning of hypothalamic circuits that control energy balance. Leptin levels are very low at birth but show a surge in release toward the second postnatal week; this surge occurs when the ARC circuits are immature (18). Mice neonates with fetal undernutrition showed a premature onset (19) and reduced surge (20) in the neonatal leptin levels, affecting the development of arcuate nucleus. Moreover, premature exogenous leptin administration in control rodents led to an accelerated weight gain (19), whereas injecting leptin into neonatal rat offspring of undernourished mothers prevented hyperphagia and excessive adiposity gain (21). However, it is important to consider species-related differences in the development of hypothalamic feeding circuits and leptin release. Indeed, in humans, serum leptin levels are high at birth and seem to fall during the first days of life (22). Bouchard *et al.* (23) suggested a biologically plausible molecular mechanism involved in the fetal programming of leptinemia in humans. They showed that fetal exposure to detrimental environments, such as impaired glucose tolerance, caused leptin gene DNA methylation adaptations and linked these findings with the increased risk of developing obesity and type 2 diabetes later in life. Findings from prospective studies in humans indicated (24) that adults with low birth weight had higher leptin concentrations than would be expected according with their degree of obesity. Unfortunately, Phillips *et al.* (24) did not ex-

amine the possible sex difference in this relationship, which hampers further comparisons with our study findings.

Previous studies have reported the gender differences in the programming effect of birth weight on health later in life (25, 26) as well as in the control of energy homeostasis (27, 28). Leptin is known to have direct effects on fetal growth and development as well as on female fertility. In women, adiposity and reproduction are intimately linked through leptin. One possible explanation of our findings therefore could be that the association between fatness and reproductive success in women may start before birth. Power and Schulkin (29) suggested that a sexual dimorphism in adiposity can be understandable, given the potential benefits to sustaining reproduction (*e.g.* fertility, lactation, age at menarche) in women and a lack of such adaptive pressures in men. The observed relationship between birth weight and leptin in females could reflect the biological importance for the development of early reproductive capacity particularly when the fetus has been exposed to an adverse intrauterine environment (30). Therefore, the ability to produce fatter girls may have reproductive benefits in terms of earlier age at menarche and increased reproductive life span (31).

Although this study includes several strengths, as its relatively large sample size and the availability of body composition data instead of simply weight or BMI to estimate adiposity gain, it should be acknowledged as a limitation that we were not able to measure estrogen levels in females, which could influence serum leptin concentrations (32). Nevertheless, the association between birth weight and serum leptin levels in females was not altered after additional adjustment for the date of the last menstruation (data not shown). Further studies in other populations, *i.e.* at birth, in childhood and at older age are warranted to confirm or refute our findings.

In conclusion, the findings observed in the present study provide further evidence for a sex-specific programming effect of birth weight on the energy homeostasis control. Our results may also contribute to explain the relationship between lower birth weight and the long-term increased risk of developing obesity and type 2 diabetes.

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