The Effect of Ponderal Index at Birth on the Relationships Between Common *LEP* and *LEPR* Polymorphisms and Adiposity in Adolescents

Idoia Labayen[1–3], Jonatan R. Ruiz[3], Luis A. Moreno[2,4], Francisco B. Ortega[3,5], Laurent Beghin[6], Stefaan DeHenauw[7], Pedro J. Benito[8], Ligia E. Diaz[9], Marika Ferrari[10], George Moschonis[11], Anthony Kafatos[12], Dénes Molnar[13], Kurt Widhalm[14], Jean Dallongeville[15], Aline Meirhaeghe[15] and Frédéric Gottrand[6]; on behalf of the HELENA study group

This study examined the effect of ponderal index (PI) at birth on the relationships between eight common polymorphisms of the leptin *(LEP)* and leptin receptor *(LEPR)* genes and adiposity in adolescents. A total of 823 European adolescents (45.4% girls) aged 14.8 ± 1.4 years were genotyped for the *LEP* (rs2167270, rs12706832, rs10244329, rs2071045, and rs3828942) and *LEPR* (rs1137100, rs1137101, and rs8179183) polymorphisms. The PI was calculated from parental reports of birth weight and length. Fat mass index (FMI) was calculated. Analyses were adjusted for relevant confounders. An "adiposity-risk-allele score" based on genotypes at the three single-nucleotide polymorphisms (SNPs) associated with adolescents' FMI in adolescents within the lower tertile of PI was calculated. The *LEP* rs10244329 and rs3828942 polymorphisms were associated with higher FMI only in adolescents within the lower PI tertile (+0.55 kg/m^2 per minor T allele, $P = 0.040$, and +0.58 kg/m^2 per major G allele, $P = 0.028$, respectively). The *LEPR* rs8179183 polymorphism was significantly associated with higher FMI in adolescents within the lower PI tertile (+0.87 kg/m^2 per minor C allele, $P = 0.006$). After correction for multiple comparisons, only the association between the *LEPR* rs8179183 and FMI persisted. However, each additional risk allele conferred 0.53 kg/m^2 greater FMI in adolescents within the lower tertile of PI ($P = 0.008$). In conclusion, our results suggest that those adolescents born with lower PI could be more vulnerable to the influence of the *LEP* rs10244329 and rs3828942 polymorphisms and *LEPR* rs8179183 polymorphism on total adiposity content. Due to the relatively small sample size, these findings should be replicated in further larger population samples.

Obesity (2011) doi:10.1038/oby.2011.74

INTRODUCTION

Obesity development and its complications are determined by lifestyle, genetic mechanisms and their interactions (1,2). Genetic factors include single-nucleotide polymorphisms (SNPs) in genes that encode proteins involved in biological processes influencing, among others, body composition or regulation of leptin, an important satiety hormone.

Epidemiological findings and data from experimental studies in animals observed associations between small body size at birth and many metabolic disorders later in life, such as type 2 diabetes (3), cardiovascular disease (4,5), hypertension (6), the metabolic syndrome (7,8), and unhealthy body composition (9,10). Inadequate nutrient supply during fetal life seems to lead to permanent changes of the structure and function of

[1]Department of Nutrition and Food Science, University of the Basque Country, Vitoria, Spain; [2]GENUD (Growth, Exercise, Nutrition and Development) Research Group, University of Zaragoza, Zaragoza, Spain; [3]Unit for Preventive Nutrition, Department of Biosciences and Nutrition at NOVUM, Karolinska Institutet, Huddinge, Sweden; [4]University School of Health Sciences, University of Zaragoza, Zaragoza, Spain; [5]Department of Medical Physiology, School of Medicine, University of Granada, Granada, Spain; [6]INSERM U995, IFR114, Faculty of Medicine, University of Lille 2, Lille, France; [7]Department of Public Health, Faculty of Medicine, University of Ghent, Ghent, Belgium; [8]Department of Health and Human Performance, Faculty of Physical Activity and Sport Sciences-INEF, Universidad Politécnica de Madrid, Madrid, Spain; [9]Immunonutrition Research Group, Department of Metabolism and Nutrition, Spanish Council for Scientific Research (CSIC), Madrid, Spain;[10]National Research Institute on Food and Nutrition (INRAN), Rome, Italy; [11]Department of Nutrition and Dietetics, Harakopio University, Athens, Greece; [12]Faculty of Medicine, University of Crete, Crete, Greece; [13]Department of Pediatrics, University of Pecs, Pecs, Hungary; [14]Division of Nutrition and Metabolism, Department of Pediatrics, Medical University of Vienna, Vienna, Austria; [15]Institut Pasteur de Lille, INSERM, U744, Lille, France. Correspondence: Idoia Labayen (idoia.labayen@ehu.es) or Luis A. Moreno (lmoreno@unizar.es)

Received 29 June 2010; accepted 14 February 2011; advance online publication 00 Month 2011. doi:10.1038/oby.2011.74

certain organs and tissues (5). These adaptations would result in an altered metabolic state, which is unfavorable in postnatal life when nutrient supply is within normal or affluent ranges.

Genetic susceptibility is important in the determination of both body size at birth and adult metabolic disorders (11–13). Ponderal index (PI) at birth is an estimate of newborn nutritional status and a proxy of the intrauterine environment that could influence gene expression leading to phenotypes associated with disease (14). Likewise, genes related to adiposity and obesity-related metabolic disorders may have different effects on individuals with different body size at birth (11,15,16).

Leptin is an adipocyte-derived hormone that suppresses food intake and increases energy expenditure by binding to and activating its specific receptor in the hypothalamus. Monogenic mutations in the leptin gene (*LEP*) and the leptin receptor gene (*LEPR*) have been shown to cause morbid obesity in mice (17–19) and humans (20,21). Human common obesity is attributed to the interaction of genes, environment, and lifestyle (22). Therefore, subtle genetic variations like polymorphisms at *LEP* and/or *LEPR* may play a significant role in the pathophysiology of human obesity. It is conceivable that common polymorphisms in the *LEP* or/and in the *LEPR* genes may modify the function or the expression of their protein products. These DNA sequence variations may cause a disruption in the leptin-signaling, contributing to common forms of human obesity.

During pregnancy, leptin is produced by maternal and fetal adipose tissue and also by the placenta, and in contrast to maternal leptin levels, umbilical cord leptin levels are positively correlated with birth weight (23). Several studies investigated the impact of SNPs in the *LEP* or *LEPR* genes on adiposity markers, but the results are not conclusive (17). In addition, the association between these SNPs and body size at birth has been little studied (24), and whether body size at birth interacts with *LEP* and *LEPR* polymorphisms and later adiposity is unknown.

The aim of the current study was to examine the effect of PI at birth on the relationship between eight common polymorphisms of the *LEP* (rs2167270, rs12706832, rs10244320, rs2071045, and rs3828942) and *LEPR* (rs1137100, rs1137101, and rs8179183) genes and adiposity in European adolescents.

METHODS AND PROCEDURES

Study design

The current report is based on data derived from the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) cross-sectional study, which aimed to obtain standardized, reliable, and comparable data from a random sample of European adolescents on a broad set of nutrition and health-related parameters (25). Data collection took place during 2006–2007 in 10 European cities: Athens and Heraklion in Greece, Dortmund in Germany, Ghent in Belgium, Lille in France, Pecs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria, and Zaragoza in Spain. A detailed description of the HELENA study sampling and recruitment approaches, standardization and harmonization processes, data collection, analysis strategies and quality control activities has been published elsewhere (25,26).

Subjects

All the adolescents meeting the general HELENA inclusion criteria (not participating simultaneously in another study, and being free of any acute infection lasting <1 week before the inclusion), with valid data for age, sex and BMI, were considered the final HELENA sample: 3,546 adolescents aged 12.5–17.5 years. In order to investigate clinical biochemistry assays and genetic analyses, one third of the adolescents were randomly selected for blood collection, resulting in a total of 1,155 adolescents. Among these participants, 823 adolescents (374 females) born at term with data on the *LEP* and *LEPR* polymorphisms, BMI and weight and length at birth were included in this study. The final sample did not differ in the main characteristics (i.e., neonatal characteristics such as body weight, length and PI at birth, age or BMI) from the original sample (all $P > 0.1$).

Written informed consent to participate was obtained from both parents and adolescents. The study was performed following the ethical guidelines of the Declaration of Helsinki 1961 (revision of Edinburgh 2000), the Good Clinical Practice, and the legislation about clinical research in humans in each of the participating countries. The protocol was approved by the Human Research Review Committee of the Universities of the centers involved (27).

Neonatal data

A questionnaire was developed for parents to collect information on the adolescents' body weight and length at birth, and duration of pregnancy (28). Parents were specifically asked to recall this information from health booklets. Duration of pregnancy was reported in two categories: between 35 and 40 weeks and >40 weeks of pregnancy. This questionnaire was sent to the parents together with the study information letter and consent form, and collected at school on the first day of the examinations. If information from the parental questionnaire was lacking, the local investigators were advised to send the questionnaire to the parents again to obtain the required information. PI was computed as birth weight (in kilograms) divided by birth length (in meters) cubed.

Physical examination

Harmonization and standardization of anthropometric measurements used to assess body composition in the HELENA study were strictly controlled and have been previously described (29). Adolescents were barefoot and in their underwear. Weight was measured with an electronic scale (Type SECA 861, SECA, Hamburg, Germany; range, 0.05–130 kg; precision, 0.05 kg), and height was measured in the Frankfurt plane with a telescopic height measuring instrument (Type SECA 225, SECA; range, 60–200 cm; precision, 1 mm). BMI was calculated as body weight in kilograms divided by the square of height in meters.

Subscapular and tricipital skinfold thicknesses were measured on the left side of the body with a Holtain caliper (range, 0–40 mm; precision, 0.2 mm) according to Lohman's anthropometric standardization reference manual (30). We used the equations reported by Slaughter *et al.* (31) to estimate body fat mass (FM).Thereafter, we calculated FM index (FMI) dividing FM by height squared (in meters), which adjust for current body size in a way comparable to that used for BMI. FMI instead of BMI was used as an indicator of adiposity as BMI has been suggested to be a less valid measurement of adiposity in adolescents and is also associated with lean mass (32).

Identification of pubertal status (stages I–V) was assessed by a medical doctor according to Marshall and Tanner (33,34). This standard staging describes breast and pubic hair development in girls and genital and pubic hair development in boys. The first stage corresponds to a prepubertal status, whereas stage V indicates a complete maturation.

Biochemistry

Serum fasting leptin concentrations were measured using the RayBio Human Leptin ELISA (enzyme-linked immunosorbent assay) (Linco Research, St Charles, MO) in duplicate. The sensitivity of leptin assay is typically <6 pg/ml, with intra- and inter-assay coefficients of variation <10% and <12%, respectively.

SNP selection and genotyping

For the *LEP* gene, we selected the entire common genetic variation (the five selected SNPs, rs2167270, rs12706832, rs10244329, rs2071045, rs3828942, captured the 11 SNPs that were reported in the HapMap database with a minor allele frequency above 0.10).

For the *LEPR* gene, the number of SNPs was very large (27 tagSNPs capturing 201 SNPs were described in the HapMap database with a minor allele frequency above 0.10) and we selected three known SNPs (rs1137100 (Arg109Lys), rs1137101 (Arg223Gln) and rs8179183 (Asn-656Lys)) based on literature data instead. These three SNPs captured by themselves 58 of the 201 reported SNPs.

The genotyping was done with an Illumina system, using the GoldenGate technology.

Statistical analysis

Linkage disequilibrium parameters ($|D'|$ and r^2) between polymorphisms were estimated using Haploview (35). To investigate the associations of *LEP* and *LEPR* polymorphisms with neonatal variables (i.e., birth weight and length, and PI), we used linear mixed models with sex and duration of pregnancy entered as covariates and center entered as random variable. Trend tests were performed by adding genotype categories in the regression analysis as ordinal variables instead of categorical variables. Likewise, we examined the association of *LEP* and *LEPR* polymorphisms with BMI, FMI, and leptin levels by using linear mixed models with sex, age, and pubertal status entered as covariates and center entered as random variable.

To examine whether the association of *LEP* and *LEPR* polymorphisms with adiposity differ by level of birth size, PI was categorized into three sex-specific tertiles as follows: <24.6 kg/m^3, 24.6–27.1 kg/m^3, and >27.1 kg/m^3 for low, middle, and upper tertile, respectively, in girls, and <24.4 kg/m^3, 24.4–27.3 kg/m^3, and >27.3 kg/m^3 for low, middle, and upper tertile, respectively, in boys. We compared genotype distributions among PI sex-specific tertiles using χ^2 tests. To test for the existence of an interaction between the polymorphisms and PI sex-specific tertiles on total adiposity, we used linear mixed models and we added a cross-product term polymorphism*PI into the model. Means of adiposity estimates were calculated by linear mixed models adjusting for pregnancy duration, sex, age, puberty stage, and center (as a random variable). Finally, an "adiposity-risk-allele score" was created by counting the total number of adiposity risk alleles across the eight variants of *LEP* and *LEPR* polymorphisms that showed interaction with PI and were associated with total adiposity content. Linear regression was used to analyze the association between the adiposity-risk-allele score and FMI across PI sex-specific tertiles. We repeated all analyses using BMI instead of FMI as adiposity estimate.

Analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 17.0 for WINDOWS; SPSS, Chicago, IL) and the level of significance was set to 0.05, except for the interaction effect, that was considered to 0.1. Data are expressed as means and standard deviation, unless otherwise stated. Finally, comparisons were adjusted for mass significance as described by Holm (36).

RESULTS

The genotypic and allelic distributions of the eight investigated SNPs, as well as genotyping success rates, are provided in **Table 1.** All SNPs presented r^2 values <0.80. The

Table 1 Genotypic and allelic distributions of the *LEP* and *LEPR* polymorphisms in the HELENA study

Gene	SNP	Genotyping success rate (%)	Genotype	Frequency (*n*, %)	MAF	*P* value H.W.
LEP	rs2167270	99.5	GG	358 (43.5)	0.34	0.92
			AG	366 (44.5)		
			AA	95 (11.5)		
	rs12706832	100	GG	276 (33.5)	0.42	0.61
			GA	398 (48.4)		
			AA	149 (18.1)		
	rs10244329	99.8	AA	223 (27.2)	0.48	0.76
			AT	414 (50.4)		
			TT	184 (22.4)		
	rs2071045	100	AA	459 (55.8)	0.25	0.42
			AG	317 (38.5)		
			GG	47 (5.7)		
	rs3828942	99.9	GG	241 (29.3)	0.48	0.94
			GA	396 (48.1)		
			AA	185 (22.5)		
LEPR	rs1137100	100	AA	490 (59.1)	0.23	0.54
			AG	286 (34.8)		
			GG	47 (5.7)		
	rs1137101	100	AA	286 (34.8)	0.42	0.15
			AG	381 (46.3)		
			GG	156 (19.0)		
	rs8179183	99.9	GG	558 (67.9)	0.18	0.49
			GC	235 (28.6)		
			CC	29 (3.5)		

H.W., Hardy–Weinberg equilibrium; HELENA, Healthy Lifestyle in Europe by Nutrition in Adolescence; LEP, leptin; LEPR, leptin receptor; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

Table 2 Descriptive characteristics of the study sample

	n	Mean	s.d.
Females	374 (45.4%)		
Males	449 (54.6%)		
Pubertal status			
Tanner stage 1 or 2	79 (10.5%)		
Tanner stage 3 or 4	456 (60.6%)		
Tanner stage 5	217 (28.9%)		
Age (years)	823	14.8	1.4
BMI (kg/m^2)	823	21.2	3.7
Body fat percentage (%)	791	23.7	9.6
Fat mass index (kg/m^2)	791	5.3	3.1
Leptin (ng/ml)	712	20.1	23.1
Neonatal data			
Birth weight (kg)	823	3.37	0.52
Birth length (cm)	823	50.6	2.6
Ponderal index at birth (kg/m^3)	823	26.03	4.16
Gestational age			
35–40 weeks	547 (66.5%)		
≥40 weeks	276 (33.5%)		

r^2 coefficients varied between 0.002 and 0.74 and between 0.07 and 0.36 for the *LEP* and *LEPR* genes, respectively (see **Supplementary Table S1** online). Genotype distributions respected the Hardy–Weinberg equilibrium ($P > 0.15$). All the SNPs had similar genotype distributions across the European centers except the *LEP* rs2071045 polymorphism ($P = 0.021$). The neonatal and clinical characteristics of adolescents are shown in **Table 2**.

Associations of *LEP* and *LEPR* polymorphisms with neonatal characteristics

There were no statistically significant differences in weight, length, and PI at birth across *LEP* and *LEPR* genotypes (see **Supplementary Table S2** online), except for the *LEPR* rs8179183 polymorphism (−0.10 kg of birth weight per minor C allele (95% confidence interval (CI): −0.16, −0.40), adjusted $P = 0.002$).

Associations of *LEP* and *LEPR* polymorphisms with total adiposity at adolescence

There were no significant differences in total adiposity estimates among genotype groups for any of the studied *LEP* polymorphisms (**Table 3**).

In contrast, adolescents carrying the A major allele of the *LEPR* rs1137100 polymorphism had higher BMI (adjusted $P = 0.015$) and FMI (adjusted $P = 0.031$) than GG adolescents (**Table 3**). The C minor allele of the *LEPR* rs8179183 polymorphism was associated with higher leptin levels (adjusted $P = 0.012$), but this relationship became nonsignificant after further adjustment for BMI (adjusted $P = 0.259$).

There were no significant interactions between *LEP* and *LEPR* polymorphisms and either sex or pubertal status (all $P > 0.1$) when considering total adiposity estimates.

Interactions between PI and *LEP* polymorphisms

The distribution of *LEP* polymorphisms was similar across the sex-specific PI tertiles (all $P > 0.2$). We did not find any significant interaction effect between the studied *LEP* polymorphisms and PI tertiles when considering leptin levels (all pregnancy duration, age, pubertal status, sex, and center-adjusted $P > 0.2$). There were significant interactions between the *LEP* rs10244329 and rs3828942 polymorphisms and PI when considering FMI (adjusted $P = 0.070$ and $P = 0.071$, respectively).

The *LEP* rs10244329 polymorphism was significantly associated with FMI (**Figure 1a**) only in adolescents with a low PI tertile (adjusted $P = 0.040$). Thus, the minor T allele of the *LEP* rs10244329 polymorphism was significantly associated with higher FMI in adolescents within the lower PI tertile (+0.55 kg/m^2 per risk allele (95% CI: 0.03, 1.07)).

Similarly, the *LEP* rs3828942 polymorphism was significantly associated with FMI (**Figure 1b**) only in adolescents belonging to the lower PI tertile (adjusted $P = 0.028$). Indeed, the major G allele of the *LEP* rs3828942 polymorphism was associated with higher FMI (+0.58 kg/m^2 per risk allele (95% CI: 0.06, 1.09)) in adolescents within the lower tertile. Nevertheless, the relationships of either the *LEP* rs10244329 or the *LEP* rs3828942 polymorphisms and FMI were attenuated and became nonsignificant after correction for multiple testing.

Both the *LEP* rs3828942 and the rs10244329 polymorphisms were significantly associated with BMI only in adolescents within the lower PI tertile (pregnancy duration, age, pubertal status, sex, and center-adjusted $P = 0.029$ and $P = 0.037$, respectively).

Interactions between PI and *LEPR* polymorphisms

The distribution of genotype variants of *LEPR* polymorphisms was similar across the sex-specific PI tertiles (all $P > 0.07$). We did not detect significant interaction effects between PI tertiles and *LEPR* polymorphisms when considering leptin levels (all $P > 0.2$). There were significant interaction effects between PI tertiles and the *LEPR* rs1137101 and rs8179183 polymorphism when considering FMI (adjusted $P = 0.094$ and $P = 0.039$, respectively), whereas no significant interaction effect was observed between the *LEPR* rs1137100 polymorphism and PI tertiles with respect to FMI (adjusted $P = 0.502$).

Adolescents within the lower PI tertile carrying the major A allele of the *LEPR* rs1137101 polymorphism showed a tendency to present higher FMI (**Figure 2a**).The *LEPR* rs8179183 polymorphism was significantly associated with FMI only in adolescents within the lower PI tertile. Thus, the minor C allele was associated with higher FMI (+0.87 kg/m^2 per minor allele (95% CI: 0.25, 1.49) adjusted $P = 0.006$) in adolescents within the lower tertile (**Figure 2b**) and this association remained significant after correction for multiple testing.

We did not find any significant relationship between the *LEPR* rs1137101 and BMI in adolescents (pregnancy duration, age, pubertal status, sex, and center-adjusted $P = 0.639$,

Table 3 Associations of the *LEP* and *LEPR* polymorphisms with adiposity in adolescents after adjusting for sex, age, pubertal status, and center

	BMI (kg/m²)		FMI (kg/m²)[a]		Leptin (ng/ml)[a]		
	Mean (s.d.)	*P* for trend	Mean (s.d.)	*P* for trend	*n*	Mean (s.d.)	*P* for trend
LEP rs2167270							
GG	21.3 (4.0)	0.505	5.2 (3.1)	0.780	270	20.1 (23.5)	0.530
GA	21.0 (3.6)		5.2 (3.0)		300	21.0 (24.4)	
AA	20.9 (3.6)		5.1 (2.8)		78	19.7 (20.6)	
LEP rs12706832							
GG	21.2 (3.6)	0.938	5.0 (2.9)	0.592	242	19.9 (23.4)	0.966
GA	21.0 (3.9)		5.2 (3.1)		341	20.6 (24.6)	
AA	21.1 (3.6)		5.5 (3.0)		130	21.3 (21.1)	
LEP rs10244329							
AA	21.0 (3.9)	0.552	4.9 (2.9)	0.376	191	19.8 (22.0)	0.703
AT	21.2 (3.8)		5.2 (3.2)		361	21.2 (24.6)	
TT	21.1 (3.4)		5.3 (2.9)		158	19.8 (23.3)	
LEP rs2071045							
AA	21.9 (4.5)	0.092	4.9 (2.9)	0.215	393	19.0 (20.2)	0.192
GA	21.4 (3.6)		5.2 (3.2)		280	22.6 (28.1)	
GG	20.9 (3.8)		5.3 (2.9)		39	19.2 (20.2)	
LEP rs3828942							
GG	21.2 (3.6)	0.460	5.3 (3.1)	0.347	208	20.3 (23.5)	0.943
GA	21.1 (3.9)		5.1 (3.0)		345	21.1 (24.5)	
AA	21.0 (3.5)		5.0 (2.9)		158	19.5 (21.7)	
LEPR rs1137100							
AA	21.2 (3.7)	0.148	5.2 (3.2)	0.062	459	21.4 (25.1)	0.117
AG	21.3 (4.0)	0.015[b]	5.2 (3.9)	0.031[b]	253	19.7 (21.6)	0.074[b]
GG	21.0 (2.8)		4.2 (2.1)		44	15.6 (17.2)	
LEPR rs1137101							
AA	21.1 (3.9)	0.452	5.2 (3.4)	0.148	269	20.1 (23.0)	0.716
AG	21.3 (3.8)		5.3 (2.8)		346	21.3 (25.1)	
GG	20.6 (3.4)		4.8 (2.7)		141	19.3 (20.7)	
LEPR rs8179183							
GG	21.0 (3.8)	0.214	5.0 (2.9)	0.059	496	19.0 (22.3)	0.096
GC	21.5 (3.5)	0.117[c]	5.5 (3.0)	0.055[c]	196	25.0 (26.5)	0.012[c]
CC	21.0 (4.5)		5.3 (4.6)		27	15.7 (19.6)	

FMI, fat mass index; leptin; LEPR, leptin receptor.
[a]Analysis was performed with log-transformed data. Leptin measurement was available in a sub-sample, so the number of subjects for this variable are presented (the number of subjects for adiposity variables are presented in **Table 1**); [b]*P* value recessive model; [c]*P* value dominant model.

$P = 0.138$, and $P = 0.437$ for the lower, middle, and upper tertiles of PI, respectively), whereas the *LEPR* rs8179183 was significantly associated with BMI only in adolescents within the lower tertile of PI (adjusted $P = 0.035$, $P = 0.493$ and, $P = 0.443$ for the lower, middle, and upper tertiles of PI, respectively).

The adiposity-risk-allele score across PI sex-specific tertiles

The adiposity-risk-allele score based on genotypes at the three SNPs associated with adolescents' FMI in adolescents born within the lower tertile of PI (*LEP* rs10244329, *LEP* rs3828942, and *LEPR* rs8179183) ranged from 0 to 3 alleles. The distribution of the adiposity-risk-allele score was similar across the sex-specific PI tertiles ($P = 0.65$). Each additional risk allele conferred an estimated 0.53 kg/m² greater FMI (95% CI: 0.14–0.93) only in adolescents within the lower tertile of PI (adjusted $P = 0.008$, $P = 0.709$, and $P = 0.335$ for the lower, middle, and upper tertiles of PI, respectively).

DISCUSSION

The present study shows that PI at birth, a proxy of the intrauterine growth, influences the associations of several common *LEP* and *LEPR* polymorphisms with adiposity in healthy

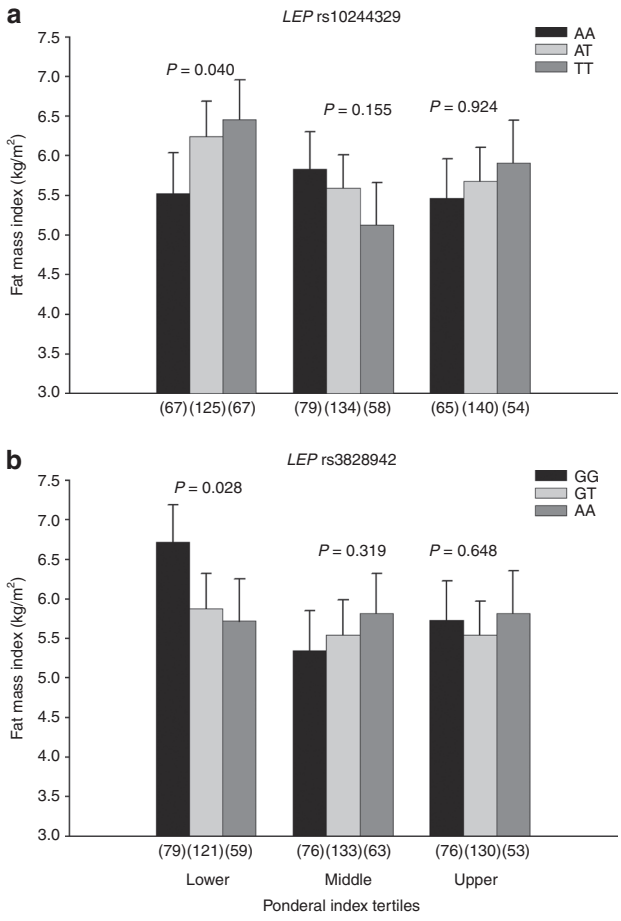

Figure 1 Interactions between leptin (*LEP*) rs10244329 and *LEP* rs3828942 and ponderal index at birth on adiposity in adolescents. Associations of the *LEP* (**a**) rs10244329 and (**b**) rs3828942 polymorphisms with fat mass index according to sex-specific tertiles of ponderal index at birth. Values are means ± standard errors adjusted for duration of pregnancy, sex, age, pubertal status, and center. Sample size in parentheses.

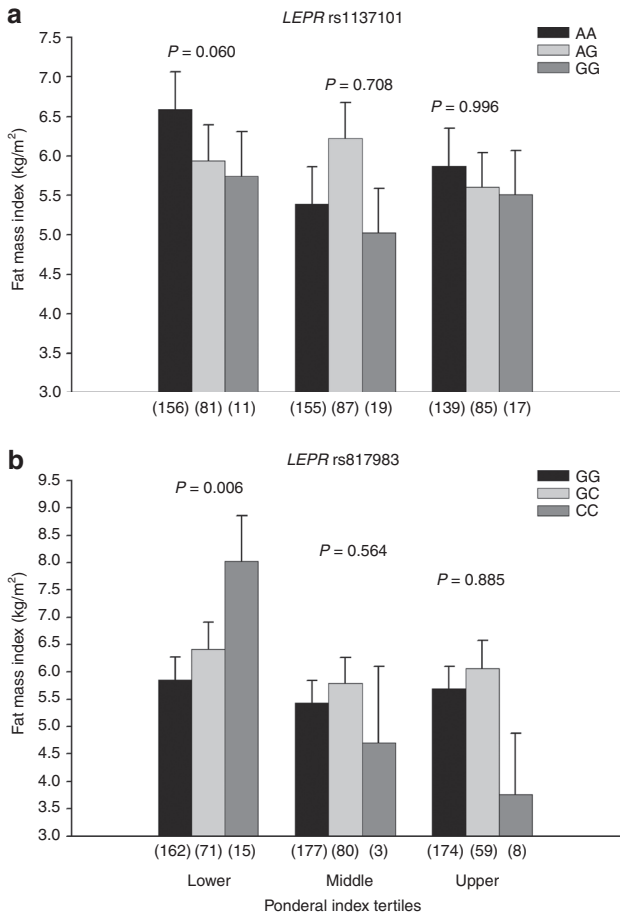

Figure 2 Interactions between leptin receptor (*LEPR*) rs1137101 and *LEPR* rs817983 and ponderal index at birth on adiposity in adolescents. Associations of the *LEPR* (**a**) rs1137101 and (**b**) rs817983 polymorphisms with fat mass index according to sex-specific tertiles of ponderal index at birth. Values are means ± standard errors adjusted for duration of pregnancy, sex, age, pubertal status, and center. Sample size in parentheses.

European adolescents. The *LEP* rs10244329 and rs3828942 polymorphisms and the *LEPR* rs8179183 polymorphisms were associated with total adiposity only in adolescents within the lower sex-specific tertile of PI, regardless of duration of pregnancy, sex, age, pubertal status, and center. To the best of our knowledge, this is the first study examining the possible interaction effect of newborn body size at birth on the association between *LEP* and *LEPR* polymorphisms and adiposity.

A number of epidemiological studies on the association of the *LEP* or *LEPR* polymorphisms with obesity and obesity-related traits have shown controversial results. Whereas several studies failed to identify any association (37–42), others found significant relationships (43–47), yet there is no consensus regarding the risk allele associated with obesity or obesity-related traits (17,39). In our study, only the rs1137100 polymorphism in the *LEPR* gene was significantly associated with total adiposity in the whole sample; adolescents carrying the A allele showed higher BMI and FMI.

The effect of common *LEP* and *LEPR* polymorphisms on neonatal characteristics has been little examined. Souren *et al.* (24), in a study conducted in monozygotic and dizygotic twins found significant and additive associations between the *LEPR* rs1137101 and rs1137100 polymorphisms and body weight at birth. They suggested a possible role for the *LEPR* gene in explaining the inverse relationship between birth weight and the development of metabolic diseases in adulthood. In our study, the *LEPR* rs1137101 and rs1137100 polymorphisms were not significantly associated with birth weight or PI at birth, even when we specifically examined those adolescents whose birth weight was below 2500 g (all adjusted $P > 0.3$). Indeed, only the *LEPR* rs8179183 polymorphism was associated with both an adiposity estimate, i.e., leptin levels, and birth weight.

The fetal origin hypothesis proposes that the nutrient and hormonal milieu of the fetus alters gene expression, causing developmental adaptations that lead to permanent changes in physiology and metabolism, which in turn can predispose to chronic diseases later in life (5). Our results suggest that individuals born with a lower PI, a surrogate of adverse intrauterine environment, could be more susceptible to be influenced by the *LEP* rs10244329 and rs3828942 polymorphisms and the *LEPR*

rs8179183 polymorphism on adiposity later in life. Therefore, we show that these three polymorphisms exert combined and additive effects on adiposity content in those adolescents born within the lower PI tertile. Nevertheless, as our sample is relatively small due to stratification on PI, larger studies or multistudy collaborations are needed to confirm these findings. The lack of studies examining the early life nutritional status effect on the relationships between these common obesity-related SNPs and adiposity later in life hampers comparisons.

We observed significant interaction effects between the *LEP* and *LEPR* polymorphisms and PI at birth, but there was no evidence for any interaction with other neonatal variables commonly used in the literature, such as birth weight or birth length. However, PI is a body proportionality index providing information about newborn's nutritional status and adiposity (48). Recent reports suggest that this index should be considered in routine growth assessment of newborns (49). PI was categorized according to sex-specific tertiles in order to maintain a relatively large numbers of participants by tertile.

The strength of the present study lays in the valid measurement of the phenotypes. Likewise, despite the analyses were restricted to 823 assessed adolescents out of 1,144 eligible subjects, the study sample did not differ significantly from the sample of those excluded neither from the study, nor from the whole sample. The sample guaranteed a large geographical spread all over Europe and all measurements followed standardized procedures throughout different study centers. Our study also had some limitations. First, when correction for multiple comparisons were made (36), most of the associations presented (relationships between the *LEP* rs10244329 and rs3828942 polymorphisms and total adiposity) would become nonsignificant. Second, the observed power for our sample size was relatively low (ranging from 0.53 to 0.77), which may have masked associations in the middle or upper PI tertiles. This warrants further investigation with larger sample sizes to draw firm conclusions. Third, other interesting data such as maternal nutrition or parental body composition indexes that may influence PI at birth and the associations studied in this report were unfortunately not recorded in the HELENA study.

In summary, the findings of the present study suggest that individuals born with a lower PI could have higher susceptibility to the deleterious effect of risk alleles of the *LEP* rs10244329 and rs3828942 polymorphisms and the *LEPR* rs8179183 polymorphism on total adiposity content later in life. Future larger studies should replicate these findings, identify the underlying mechanisms and assess whether these effects persist into adulthood.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/oby

ACKNOWLEDGMENTS

The HELENA study was carried out with the financial support of the European Community Sixth RTD Framework Programme (Contract FOODCT-2005-007034). Complete list of researchers of HELENA study group can be found in http://www.helenastudy.com/list.php. This work was also partially supported by the Swedish Council for Working Life and Social Research (FAS), and by grants from the Spanish Ministry of Education (EX-2007-1124), the Spanish Ministry of Health: Maternal, Child Health and Development Network (number RD08/0072) and the University of the Basque Country, and Grants from the Spanish Ministry of Education (EX-2008-0641), the Spanish Ministry of Science and Innovation (RYC-2010-05957) and the Swedish Heart-Lung Foundation (20090635). The writing group takes sole responsibility for the content of this article. The content of this paper reflects only the authors' views, and the European Community is not liable for any use that may be made of the information contained therein. None of the authors had any personal or financial conflict of interest.

DISCLOSURE

The authors declared no conflict of interest.

REFERENCES

1. Thorleifsson G, Walters GB, Gudbjartsson DF *et al*. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet* 2009;41:18–24.
2. Loos RJ, Rankinen T. Gene-diet interactions on body weight changes. *J Am Diet Assoc* 2005;105:S29–S34.
3. Martin-Gronert MS, Ozanne SE. Programming of appetite and type 2 diabetes. *Early Hum Dev* 2005;81:981–988.
4. Labayen I, Ortega FB, Sjöström M, Ruiz JR. Early life origins of low-grade inflammation and atherosclerosis risk in children and adolescents. *J Pediatr* 2009;155:673–677.
5. Barker DJ. The developmental origins of adult disease. *J Am Coll Nutr* 2004;23:588S–595S.
6. Alexander BT. Fetal programming of hypertension. *Am J Physiol Regul Integr Comp Physiol* 2006;290:R1–R10.
7. Labayen I, Ruiz JR, Vicente-Rodríguez G *et al*. Early life programming of abdominal adiposity in adolescents: The HELENA Study. *Diabetes Care* 2009;32:2120–2122.
8. Ramadhani MK, Grobbee DE, Bots ML *et al*. Lower birth weight predicts metabolic syndrome in young adults: the Atherosclerosis Risk in Young Adults (ARYA)-study. *Atherosclerosis* 2006;184:21–27.
9. Labayen I, Moreno LA, Blay MG *et al*. Early programming of body composition and fat distribution in adolescents. *J Nutr* 2006;136:147–152.
10. Labayen I, Moreno LA, Ruiz JR *et al*. Small birth weight and later body composition and fat distribution in adolescents: the Avena study. *Obesity (Silver Spring)* 2008;16:1680–1686.
11. Gielen M, Lindsey PJ, Derom C *et al*. Modeling genetic and environmental factors to increase heritability and ease the identification of candidate genes for birth weight: a twin study. *Behav Genet* 2008;38:44–54.
12. Pulizzi N, Lyssenko V, Jonsson A *et al*. Interaction between prenatal growth and high-risk genotypes in the development of type 2 diabetes. *Diabetologia* 2009;52:825–829.
13. Meirhaeghe A, Boreham CA, Murray LJ *et al*. A possible role for the PPARG Pro12Ala polymorphism in preterm birth. *Diabetes* 2007;56:494–498.
14. Junien C, Nathanielsz P. Report on the IASO Stock Conference 2006: early and lifelong environmental epigenomic programming of metabolic syndrome, obesity and type II diabetes. *Obes Rev* 2007;8:487–502.
15. Labayen I, Moreno LA, Marti A *et al*. Effect of the Ala12 allele in the PPARgamma-2 gene on the relationship between birth weight and body composition in adolescents: the AVENA study. *Pediatr Res* 2007;62:615–619.
16. Ruiz JR, Labayen I, Ortega FB *et al*. Birth weight and blood lipid levels in Spanish adolescents: influence of selected APOE, APOC3 and PPARgamma2 gene polymorphisms. The AVENA Study. *BMC Med Genet* 2008;9:98.
17. Paracchini V, Pedotti P, Taioli E. Genetics of leptin and obesity: a HuGE review. *Am J Epidemiol* 2005;162:101–114.
18. Oswal A, Yeo GS. The leptin melanocortin pathway and the control of body weight: lessons from human and murine genetics. *Obes Rev* 2007;8:293–306.
19. Woods AJ, Stock MJ. Leptin activation in hypothalamus. *Nature* 1996;381:745.
20. Beckers S, Zegers D, Van Gaal LF, Van Hul W. The role of the leptin-melanocortin signalling pathway in the control of food intake. *Crit Rev Eukaryot Gene Expr* 2009;19:267–287.

21. Farooqi IS, O'Rahilly S. Mutations in ligands and receptors of the leptin-melanocortin pathway that lead to obesity. *Nat Clin Pract Endocrinol Metab* 2008;4:569–577.
22. Yiannakouris N, Yannakoulia M, Melistas L *et al*. The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability. *J Clin Endocrinol Metab* 2001;86:4434–4439.
23. Schulz S, Häckel C, Weise W. Hormonal regulation of neonatal weight: placental leptin and leptin receptors. *BJOG* 2000;107:1486–1491.
24. Souren NY, Paulussen AD, Steyls A *et al*. Common SNPs in LEP and LEPR associated with birth weight and type 2 diabetes-related metabolic risk factors in twins. *Int J Obes (Lond)* 2008;32:1233–1239.
25. De Henauw S, Gottrand F, De Bourdeaudhuij I *et al*. Nutritional status and lifestyles of adolescents from a public health perspective. The HELENA Project—Healthy Lifestyle in Europe by Nutrition in Adolescence. *J Public Health* 2007;15:187–197.
26. Moreno LA, De Henauw S, González-Gross M *et al*. Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. *Int J Obes (Lond)* 2008;32 Suppl 5:S4–11.
27. Béghin L, Castera M, Manios Y *et al*. Quality assurance of ethical issues and regulatory aspects relating to good clinical practices in the HELENA Cross-Sectional Study. *Int J Obes (Lond)* 2008;32 Suppl 5:S12–S18.
28. Iliescu C, Béghin L, Maes L *et al*. Socioeconomic questionnaire and clinical assessment in the HELENA Cross-Sectional Study: methodology. *Int J Obes (Lond)* 2008;32 Suppl 5:S19–S25.
29. Nagy E, Vicente-Rodriguez G, Manios Y *et al*. Harmonization process and reliability assessment of anthropometric measurements in a multicenter study in adolescents. *Int J Obes (Lond)* 2008;32 Suppl 5:S58–S65.
30. Lohman TG, Roche AF, Martorell R. *Anthropometric Standardization Reference Manual*. Human Kinetics: Champaign, IL, 1991.
31. Slaughter MH, Lohman TG, Boileau RA *et al*. Skinfold equations for estimation of body fatness in children and youth. *Hum Biol* 1988;60: 709–723.
32. Wells JC, Williams JE, Chomtho S *et al*. Pediatric reference data for lean tissue properties: density and hydration from age 5 to 20 y. *Am J Clin Nutr* 2010;91:610–618.
33. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child* 1969;44:291–303.
34. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 1970;45:13–23.
35. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–265.
36. Holm S. A simple sequentially rejective multiple test procedure. *Scand J Statist* 1979;6:65–70.
37. Constantin A, Costache G, Sima AV *et al*. Leptin G-2548A and leptin receptor Q223R gene polymorphisms are not associated with obesity in Romanian subjects. *Biochem Biophys Res Commun* 2010;391: 282–286.
38. Endo K, Yanagi H, Hirano C *et al*. Association of Trp64Arg polymorphism of the beta3-adrenergic receptor gene and no association of Gln223Arg polymorphism of the leptin receptor gene in Japanese schoolchildren with obesity. *Int J Obes Relat Metab Disord* 2000;24:443–449.
39. Heo M, Leibel RL, Fontaine KR *et al*. A meta-analytic investigation of linkage and association of common leptin receptor (LEPR) polymorphisms with body mass index and waist circumference. *Int J Obes Relat Metab Disord* 2002;26:640–646.
40. Matsuoka N, Ogawa Y, Hosoda K *et al*. Human leptin receptor gene in obese Japanese subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity. *Diabetologia* 1997;40:1204–1210.
41. Portolés O, Sorlí JV, Francés F *et al*. Effect of genetic variation in the leptin gene promoter and the leptin receptor gene on obesity risk in a population-based case-control study in Spain. *Eur J Epidemiol* 2006;21:605–612.
42. Meirhaeghe A, Cottel D, Amouyel P, Dallongeville J. Lack of association between certain candidate gene polymorphisms and the metabolic syndrome. *Mol Genet Metab* 2005;86:293–299.
43. Furusawa T, Naka I, Yamauchi T *et al*. The Q223R polymorphism in LEPR is associated with obesity in Pacific Islanders. *Hum Genet* 2010;127: 287–294.
44. Chagnon YC, Chung WK, Pérusse L *et al*. Linkages and associations between the leptin receptor (LEPR) gene and human body composition in the Québec Family Study. *Int J Obes Relat Metab Disord* 1999;23:278–286.
45. Duarte SF, Francischetti EA, Genelhu VA, Cabello PH, Pimentel MM. LEPR p.Q223R, beta3-AR p.W64R and LEP c.-2548G>A gene variants in obese Brazilian subjects. *Genet Mol Res* 2007;6:1035–1043.
46. Guízar-Mendoza JM, Amador-Licona N, Flores-Martínez SE *et al*. Association analysis of the Gln223Arg polymorphism in the human leptin receptor gene, and traits related to obesity in Mexican adolescents. *J Hum Hypertens* 2005;19:341–346.
47. Mattevi VS, Zembrzuski VM, Hutz MH. Association analysis of genes involved in the leptin-signaling pathway with obesity in Brazil. *Int J Obes Relat Metab Disord* 2002;26:1179–1185.
48. Moyer-Mileur LJ, Slater H, Thomson JA *et al*. Newborn adiposity measured by plethysmography is not predicted by late gestation two-dimensional ultrasound measures of fetal growth. *J Nutr* 2009;139:1772–1778.
49. Olsen IE, Lawson ML, Meinzen-Derr J *et al*. Use of a body proportionality index for growth assessment of preterm infants. *J Pediatr* 2009;154: 486–491.