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Reference values for leptin, cortisol, insulin and glucose, among European adolescents and their association with adiposity: The HELENA Study

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Abstract

Background and Objective: **Adequate concentrations of leptin, cortisol, and insulin are important for a suitable metabolism and development during adolescence. These hormones jointly with glucose play a major role in fat metabolism and development of childhood obesity. Our main objective was to quantify biomarkers as leptin, cortisol, insulin and glucose status in European adolescents to contribute to establish reference ranges.**

Methods: **A representative sample of 927 adolescents (45% males, 14.9±1.2 years for the overall population) from ten European cities of the HELENA study was used to obtain fasting blood samples for these biomarkers. The percentile distributions were computed by sex and age and percentiles were associated with BMI classification.**

Results: **Serum leptin concentration in adolescents varied significantly according to BMI, sex and age (all p<0.001). Cortisol presented a tendency to increase with age, both for females and males, while insulin and glucose were stable with age. Leptin and insulin were highest in obese adolescents (p<0.001), whilst cortisol and glucose did not vary with BMI. Percentiles 5, 25, 50, 75 and 95,**

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VALORES DE REFERENCIA PARA LEPTINA, CORTISOL, INSULINA Y GLUCOSA ENTRE LOS ADOLESCENTES EUROPEOS Y SU ASOCIACIÓN CON ADIPOSIDAD: ESTUDIO HELENA

Resumen

Objetivo: **Concentraciones adecuadas de leptina, cortisol e insulina son importantes para un metabolismo normal durante la adolescencia, puesto que valores alterados de estas hormonas, junto con la glucosa, se asocian con el desarrollo de la obesidad infantil. Nuestro principal objetivo fue cuantificar estos marcadores en adolescentes europeos con el fin de establecer rangos de referencia.**

Métodos: **Muestras de sangre procedentes de 927 adolescentes en ayunas (14,9 ± 1,2 años, 45% varones, estudio HELENA), fueron analizadas para cuantificar la leptina, cortisol, insulina y glucosa. Las distribuciones de percentiles se determinaron teniendo en cuenta el sexo y la edad. También se estudió la asociación entre percentiles y la clasificación del IMC.**

Resultados: **La concentración de leptina en suero variaba significativamente con el IMC, el sexo y la edad (todos p<0,001). El cortisol presentó una tendencia a aumentar con la edad, tanto para varones como mujeres, mientras que la insulina y la glucosa eran estables con la edad. La leptina y la insulina fueron más altas en los adolescentes obesos (p <0,001), mientras que el cortisol y glucosa no variaron con el IMC. Los percentiles 5, 25, 50, 75 y 95, para los valores de hormonas fueron, respectivamente: 1.27, 4.06, 11.54, 26.70 y 65.33 ng/ml para la leptina; 5.00, 8.11, 11.14, 15.00 y 24.51 μg/dl para el cortisol y 3.65, 6.15, 8.52, 11.90 y 20.53 μIU/ml de insulina.**

for hormones values were, respectively: 1.27, 4.06, 11.54, 26.70 and 65.33 ng/ml for leptin; 5.00, 8.11, 11.14, 15.00 and 24.51 μg/dl for cortisol and 3.65, 6.15, 8.52, 11.90 and 20.53 μIU/ml for insulin.

Conclusions: **In adolescents, leptin, cortisol, insulin and glucose concentrations are differently affected by age, sex and BMI. Establishment of reference ranges (percentiles) of these biomarkers would be of great interest when pediatricians have to assess the trend of an adolescent to develop obesity years after.**

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Key words: *Leptin. Cortisol. Insulin. Glucose. Adiposity and Adolescents.*

Conclusiones: **En los adolescentes, las concentraciones de referencia de leptina, cortisol, insulina y glucosa se ven afectados de manera diferente según la edad, el sexo y el IMC.**

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Palabras claves: *Leptina. Cortisol. Insulina. Glucosa. Adiposidad y adolescentes.*

Introduction

Since leptin was identified in 1994, hormones participating in energy homeostasis control have gained on importance, especially regarding the current obesity epidemic[1]. Nowadays it is known that adipocytes produce a number of substances and hormones apart from leptin, which interfere in energetic balance, as an example adiponectin, that acts cooperatively with insulin for glucose uptake[2].

Besides the substances produced by adipocytes, other components of the endocrine system could be associated with adiposity and organic homeostasis. Cortisol, synthesized by the adrenal cortex, for example, could influence leptin levels due to the possible leptin secretion reduction under stress conditions. In fact, high serum cortisol levels in the morning have been associated with metabolic syndrome in overweight and obese children and adolescents[3]. Available evidences suggest that leptin has inhibitory role on insulin secretion and serum leptin concentration has been proposed as an important predictor of insulin resistance and other metabolic risks irrespective of obesity levels in adults[4]. All three (leptin, insulin and cortisol) are implicated in fatty acids metabolism and inflammation. Several studies have demonstrated a quantitative relation between higher concentrations of these hormones and the development of obesity as well as their complications[2].

Generally, values of leptin, cortisol, insulin and glucose could be altered in the pediatric population with overweight and their comorbidities. However, reference values for healthy population are limited, hindering to answer questions such as: Is BMI associated with values of cortisol, glucose and insulin in healthy adolescents, as demonstrated for leptin? Do these substances vary with sex and age? Could reference curves be created in order to help assessment of adolescents in clinical practice? In this way, the main objective of the present study was to describe leptin, cortisol, insulin and glucose status in adolescents, contributing to establish reference values, which nowadays are not available for this population.

Subjects and Methods

Subjects, recruitment and study design

The HELENA-CSS (Healthy Lifestyle in Europe by Nutrition in Adolescence) study is a multi-centre cross-sectional study on lifestyle and nutrition among adolescents, from 10 European cities from nine different countries: Athens and Heraklion (Greece), Dortmund (Germany), Ghent (Belgium), Lille (France), Pecs (Hungary), Rome (Italy), Stockholm (Sweden), Vienna (Austria), and Zaragoza (Spain). Inclusion criteria were: being 12.5-17.5 years old, not participating simultaneously in another clinical trial and free of any acute infection earlier occurring than one week before inclusion[5]. Participants were recruited by a multi-stage random cluster sampling procedure, using schools as primary sampling units and classes as secondary sampling units. The sample size was calculated according to stratified random sampling with proportional affixation to the size of the strata (SEX and AGE) and minimum variance under Neyman allocation. A confidence level of 95% and a minimum of ± 0.3 error for body mass index (BMI) were chosen. On city level, diversity of the sample with respect to cultural and socio-economic aspects was achieved by performing a random proportional distribution of all schools taking into account the site (district/zone of the city) and the type of school (public or private). The complete description of the design and implementation of the study has been described elsewhere[6]. The study has been performed following the ethical guidelines of the Declaration of Helsinki 1964 (revision of Edinburgh 2000), Convention of Oviedo (1997), the Good Clinical Practice, and the legislation about clinical research in humans in each of the participating countries. Informed written consent was obtained from all participants and parents or guardians[7].

Anthropometric measurements

Adolescents' height and weight were measured by trained researchers in a standardized way. The weight

was recorded to the nearest 0.1kg, using an electronic scale (Type SECA 861, UK). The height was recorded to the nearest 0.1 cm, using a telescopic height measuring instrument (Type SECA 225, UK). The complete description of the anthropometric measurements of the study has been described elsewhere[6]. The Body Mass Index (BMI) of the adolescents was calculated from their measured height and weight (BMI = weight divided by height squared, [kg/m²]). The sample was classified in underweight, normal weight, overweight and obese, according to the international gender and age-specific BMI cut-off points proposed by Cole et al[8]. These points have been established for children and adolescents aged from 2 to < 18 years old separately for males and females. These cut-off values are based on percentiles of adults (>18 years) considering BMI <18.5 Kg/m^2 for underweight, >25 Kg/m^2 for overweight and 30kg/m^2 for obesity[8].

Tanner Stage was used for the description of adolescents' pubertal maturity. For the determination of Tanner Stage, physical examination was performed by a physician aiming to classify the adolescents into one of the five stages of pubertal maturity defined by Tanner et al.[9].

Specimen collection and biochemical analyses

Blood sampling was randomly performed in one third of the recruited adolescents due to the low variability of biochemical markers. For this study was considered a subsample of 927 adolescents (421 males and 506 females) who completed the blood sampling for leptin, cortisol, insulin and glucose, with a mean age of 14.9 ±1.2 years. Fasting blood samples were collected by venipuncture at school between eight and ten o'clock in the morning between November 2006 and October 2007, during the whole academic year, excluding summer period. The complete sampling protocol has been published elsewhere[10].

Leptin, cortisol, insulin and glucose assessment

For the measurements of glucose, leptin and cortisol blood was collected in serum tubes and centrifugated at 3500 rpm for 15 min. For insulin, heparin plasma was collected, put immediately on ice and centrifugated at 3500rpm for 15 min. The heparin plasma samples were transported under cooled conditions to the Central Laboratory in Bonn (Germany) and frozen at -80 ºC. Insulin was analysed at the Nutrition and Food Science laboratory from the University of Bonn by immunoassay (Immulite 2000, DPC Biermann GmbH, Bad Nauheim, Germany). Serum aliquots were transported at stable room temperature and frozen at -80°C afterwards. Glucose was measured enzymatically on the Dimension RxL clinical chemistry system (Dade Behring, Schwalbach, Germany) in fresh serum samples at the University of Bonn. Samples of leptin and cortisol were analysed at the Biochemical Laboratory of the Faculty of Physical Activity and Sport Sciences (INEF) UPM, Madrid (Registered Lab number 242, Red de Laboratorios de la Comunidad de Madrid). Concentration of serum leptin (ng/mL) was measured using the RayBio® Human Leptin ELISA (Enzyme-Linked Immunosorbent Assay) kit. The sensitivity of leptin assay was less than 6 pg/mL, with intra and inter-assay coefficients of variation of <10% and <12%. To determine cortisol, a fluorescence polarisation enzymoassay in the AxSYM analyser was used (Abbott Diagnostics, Illinois, U.S.A.).

Statistical analysis

Cortisol, insulin, leptin showed non-normal distribution while glucose presented a normal distribution and the residuals showed a satisfactory pattern. Descriptive statistics were performed and values are shown as mean, standard deviation, percentile, median, minimum and maximum. The differences between sex, age groups and BMI groups were analysed using one-way ANOVA. All the analyses were adjusted by means of a weighting factor to balance the sample according to the age and sex distribution of the theoretical sample, to guarantee representation of each of the stratified groups. The *Pearson´s* correlation was performed to evaluate the correlation between BMI, and hormones concentrations. To provide percentile value curves for European adolescents, leptin, cortisol, insulin and glucose data were analysed by maximum penalised likelihood using the least mean square statistical method for boys and girls separately. Smoothed centile charts using the least mean square method were derived. This estimates the measurement centiles in terms of three age–sex-specific cubic spline curves: the L curve (Box–Cox power to remove skewness), M curve (median) and S curve (CV). For the construction of the percentile curves, data were imported into the LMS ChartMaker software (version 2.3; by Tim Cole and Huiqi Pan, HarlowHealthcare, South Shields, Tyne and Wear, UK) and the L, M and S curves were estimated. The rest of the data were analysed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $p<0.05$.

Results

General descriptive characteristics from the study sample, including mean leptin, cortisol, insulin and glucose concentrations are presented in table I. Significant differences between genders were only observed in mean leptin values, females had higher values than males ($p<0.001$). Most of the adolescents had normal weight (72.7%; 674/927), 5.9% (55/927) underweight, 16.9% (157/927) overweight and 4.4% (41/927) obe-

Table I
Descriptive characteristics of participants (mean values ± SD, minimum and maximum)

	All *Mean ± SD* *(Min – Max)*	*Males* *Mean ± SD* *(Min – Max)*	*Females* *Mean ± SD* *(Min – Max)*	*P* *value*
N	927	421	506	
Age (yr)	14.85 ± 1.23 (12.50 – 17.42)	14.83 ± 1.26 (12.50 – 17.33)	14.87 ± 1.20 (12.50 – 17.42)	ns
Tanner stages I/II/III/IV/V (%)	1/6/19/44/32	1/7/18/43/31	0/4/20/44/32	ns
BMI (Kg/m^2)	21.27 ± 3.50 (14.15 – 35.80)	21.24± 3.67 (14.15 – 35.82)	21.30 ± 3.36 (14.21 –34.37)	ns
Leptin (ng/ml)	19.44 ± 22.16 (0.18 – 169.97)	9.07 ± 13.76 (0.18 – 93.04)	28.06± 24.06 (1.39 – 169.97)	<0.001
Cortisol (µg/dl)	12.42 ± 6.41 (2.00 – 50.00)	11.88 ± 5.14 (2.00 – 35.00)	12.88 ± 7.28 (3.00 – 50.00)	ns
Insulin (µlU/ml)	10.13 ± 7.66 (1.99 –90.60)	10.05 ± 8.81 (1.99 – 90.6)	10.20 ± 6.57 (1.99 – 84.6)	ns
Glucose (mg/dl)	90.90 ± 7.29 (55.00 – 135.00)	92.86 ± 7.40 (55.00 – 135.00)	89.28 ± 6.79 (68.00 – 120.00)	ns

ns = $P > 0.05$

sity. Most adolescents (70%) were classified as Tanner stages 4 or 5, but there were no gender differences.

For leptin, cortisol, insulin and glucose concentrations, percentile distribution by age and gender are shown in tables II-V and smoothed centile curves (P5, P25, P50, P75, P95), studied by age and gender are presented in figure 1.

In males, leptin concentrations show a decreasing tendency with increasing age. On the other hand, females have no variation in leptin concentrations by age. For both gender a significant increasing cortisol concentration was observed with increasing age (table III). Otherwise, insulin values decrease by increasing age in females and males (Table IV). As presented in table V, glucose concentration was quite stable, with an increasing tendency in both mean and median values with age.

Associations between leptin, cortisol, insulin and glucose values and BMI.

Figure 2 shows the hormonal values and glucose concentration according to the BMI classifications:

Table II
Percentiles of leptin concentrations by age and sex in European adolescents

	Leptin levels (ng/ml)											
	Mean	*SD*	*P2.5*	*P5*	*P10*	*P25*	*P50*	*P75*	*P90*	*P95*	*P97.5*	*P100*
Total (n 927)	19.44	22.16	0.97	1.27	1.79	4.06	11.54	26.70	49.11	65.33	78.76	169.97
Male (n 421)												
age ≤13 (years)	12,51	17,24	1,16	1,48	1,80	3,03	5,49	11,36	34,62	56,33	65,34	85,20
age 14 (years)	10,35	16,21	0,84	0,93	1,19	1,84	3,68	12,41	24,88	53,07	61,68	93,04
age 15 (years)	5,61	5,38	0,40	0,68	0,97	1,77	3,95	7,65	13,33	16,84	21,21	29,12
age≥16 (years)	8,26	11,45	0,64	0,97	1,31	1,79	3,67	8,99	23,85	35,63	53,07	56,40
Female (n 506)												
age ≤13 (years)	25,81	21,11	2,93	3,84	5,86	10,10	19,07	33,47	59,24	69,63	76,60	106,63
age 14 (years)	30,65	26,39	4,07	4,70	6,49	13,36	22,75	38,61	72,36	84,62	116,12	127,75
age 15 (years)	26,59	19,36	3,02	3,70	6,28	11,70	21,94	38,36	57,02	65,76	71,73	90,51
age ≥16 (years)	31,18	30,18	2,31	3,56	6,05	11,22	22,29	39,56	64,85	79,83	133,94	169,97

Table III
Percentiles of cortisol concentrations by age and sex in European adolescents

	Cortisol levels (µg/dl)											
	Mean	*SD*	*P2.5*	*P5*	*P10*	*P25*	*P50*	*P75*	*P90*	*P95*	*P97.5*	*P100*
Total (n 927)	12.42	6.41	4.50	5.00	5.90	8.10	11.14	15.00	20.00	24.51	30.03	50.00
Male (n 421)												
age 13 (years)	10,69	4,84	3,50	4,00	5,30	7,30	10,00	13,40	16,80	20,40	23,70	27,30
age 14 (years)	11,26	4,79	4,10	5,00	5,90	7,50	10,60	14,10	18,30	19,50	20,20	34,10
age 15 (years)	11,81	4,87	4,90	5,30	6,20	8,00	10,85	14,75	19,10	20,50	22,80	27,70
age 16 (years)	14,42	5,37	5,70	6,40	8,00	11,10	13,80	17,10	22,10	23,60	30,00	34,50
Female (n 506)												
age 13 (years)	11,10	5,62	4,20	4,80	5,30	7,30	9,90	13,40	18,30	23,00	24,80	34,70
age 14 (years)	11,75	4,97	4,50	5,00	6,00	8,20	11,00	14,20	18,30	22,10	23,00	30,50
age 15 (years)	13,20	7,43	4,40	4,80	5,40	8,10	11,60	15,80	23,30	30,70	33,20	44,10
age 16 (years)	15,90	9,74	5,50	6,00	7,10	9,10	12,40	19,80	32,10	38,10	40,30	50,00

underweight, normal weight, overweight and obese. Leptin shows a significant progressive and linear increase according to BMI, being both positively correlated ($p<0.001$). According to the multivariate comparison model, with Bonferroni correction, the leptin levels for each BMI classification were significantly different ($p<0.001$). The highest leptin levels were observed in obese adolescents. Similar to leptin, insulin concentrations presented a progressively increasing concentration according to BMI, being both positively correlated ($p<0.001$). Insulin values for obese adolescents were significantly higher, from those classified as overweight, normal weight and underweight ($p<0.001$).

For both males and females a positive correlation between leptin and insulin was observed ($r=0.409$ and 0.304; $p<0.001$). Cortisol and glucose showed no variation of the levels according to BMI classification, but a positive correlation was observed between glucose and cortisol for males ($r=0.293$; $p<0.001$). Glucose presented a positive correlation with insulin in both gender ($p<0.001$).

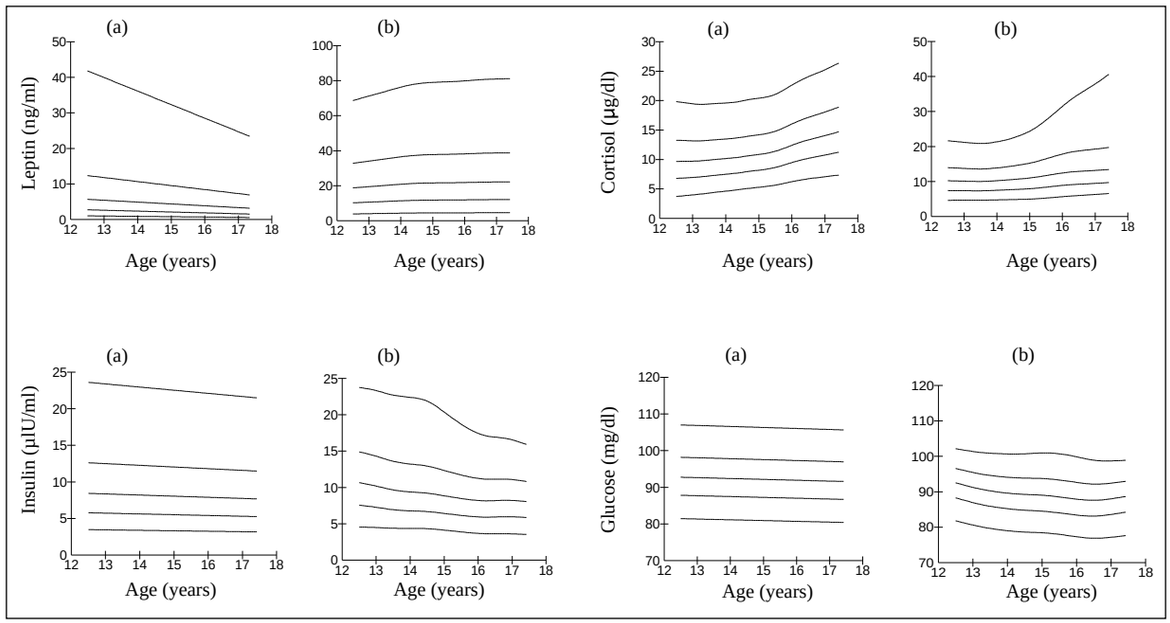

Fig. 1.—Smoothed centile curves (from the bottom to the top: P5, P25, P50, P75, P95) of leptin (ng/ml), cortisol (µg/dl), insulin (µIU/ml) and glucose (mg/dl) in (a) males and (b) females.

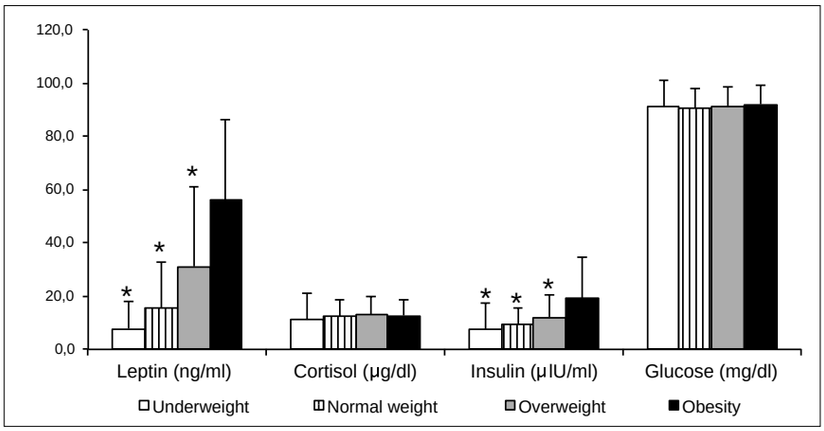

*Fig. 2.—Leptin, cortisol, insulin and glucose concentrations by BMI in European adolescents (mean values and standard deviations). *(p<0.01).*

Discussion

To the best of our knowledge, the data obtained in the framework of the HELENA study are the first descriptive values for establishing leptin, cortisol, insulin and glucose levels in apparently healthy European adolescents.

Our results showed that serum leptin concentration in adolescents varied significantly according to BMI, sex and age. The scientific interest on leptin concentrations in adolescents and pre-pubertal population has been increasing since the relation of high leptin values with an increased risk of insulin resistance is known[11,12]. In pediatric studies,higher values in obese subjects are related to inflammation and possibly to the metabolic syndrome[13]. Our results revealed a positive correlation between leptin concentration and BMI in both sexes. Leptin values among boys did not show significant variation with age; on the other hand, among girls, leptinemia increases according to increasing age as described earlier[14]. Cross-sectional studies have shown the presence of a significant sexual dimorphism in circulating leptin concentrations during puberty with higher values in girls independently of BMI, which is comparable with data found in our study [12,15,16,17,18,19,20]. However, it should be taken into account that during puberty boys and girls have different changes in body composition, which also influence leptin levels[18]. Estradiol has a stimulatory effect on leptin concentration in females, while testosterone has a suppressive effect[21]. Furthermore, some studies[22,23] showed a persistent gender difference in leptin concentrations even after adjustment for differences in testosterone, estradiol and percentage of fat mass in a group of children and adolescents. Findings of this study indicate that other metabolic or hormonal sex-related variables could probably influence leptin production[23].

To evaluate reference values related to glucose, we used the normal ranges for fasting glucose clas-

Table IV
Percentiles of insulin concentration by age and sex in European adolescents

	Insulin levels (µlU/ml)											
	Mean	*SD*	*P2.5*	*P5*	*P10*	*P25*	*P50*	*P75*	*P90*	*P95*	*P97.5*	*P100*
Total (n 927)	10.13	7.66	2.76	3.65	4.58	6.15	8.52	11.90	16.20	20.53	26.55	90.60
Male (n 421)												
age 13 (years)	10,90	8,50	2,02	3,08	3,67	5,90	8,32	12,10	21,10	28,70	37,70	51,30
age 14 (years)	10,60	10,30	2,22	3,76	4,58	6,23	8,75	11,80	15,50	19,80	30,80	90,60
age 15 (years)	9,60	9,00	2,49	3,47	4,49	5,92	7,96	11,10	14,80	19,70	26,40	90,20
age 16 (years)	9,10	6,30	2,62	3,28	3,88	5,68	7,40	11,00	14,50	18,30	26,40	48,40
Female (n 506)												
age 13 (years)	11,40	6,50	3,88	4,19	5,30	6,97	9,96	14,20	19,70	22,40	28,60	45,80
age 14 (years)	11,00	9,10	3,18	4,90	5,48	6,75	9,43	12,30	16,20	21,00	23,00	84,60
age 15 (years)	9,50	4,30	3,52	3,84	4,71	6,68	8,65	11,50	15,30	17,30	22,40	24,90
age 16 (years)	8,80	4,20	2,69	3,86	4,41	5,53	8,14	11,30	14,40	17,00	17,40	27,90

Table V
Percentiles of glucose concentrations by age and sex in European adolescents

	Glucose levels (mg/dl)											
	Mean	*SD*	*P2.5*	*P5*	*P10*	*P25*	*P50*	*P75*	*P90*	*P95*	*P97.5*	*P100*
Total (n 927)	90.90	7.29	77.00	79.26	82.00	86.00	91.00	95.00	100.00	103.00	105.00	135.00
Male (n 421)												
age 13 (years)	91,73	9,12	73,00	79,00	80,00	87,00	91,00	93,00	102,00	111,00	113,00	113,00
age 14 (years)	92,54	7,25	79,00	81,00	84,00	88,00	92,00	97,00	101,00	104,00	106,00	135,00
age 15 (years)	93,74	7,00	79,00	82,00	85,00	90,00	93,00	99,00	103,00	105,00	106,00	117,00
age 16 (years)	93,31	7,74	75,00	79,00	80,00	91,00	94,00	98,00	101,00	104,00	104,00	108,00
Female (n 506)												
age 13 (years)	90,90	5,67	81,00	81,00	86,00	88,00	90,00	93,00	99,00	103,00	103,00	109,00
age 14 (years)	89,11	6,90	76,00	78,00	81,00	85,00	89,00	94,00	98,00	100,00	103,00	120,00
age 15 (years)	89,10	7,13	77,00	77,00	80,00	83,00	90,00	93,00	99,00	101,00	103,00	106,00
age 16 (years)	90,17	5,50	75,00	83,00	84,00	86,00	90,00	93,00	97,00	98,00	101,00	101,00

sified by Di Bonito et al.[24]. These authors performed a classification in order to evaluate the association between fasting plasma glucose and cardio metabolic risk factors. Their classifications for normal ranges of glucose are: low normal (≤82 mg/dl), mid-normal (83–88 mg/dl), and high-normal (89–99 mg/dl). The values expressed by these authors were considered in our evaluation since children and adolescents with high-normal glucose presented a higher risk of insulin resistance, hypertension, elevated white blood cell values than those with low normal glycemic, independent of BMI[24]. In this sense, the interpretation for the glucose values distribution in percentiles of the present study indicates that the adolescents up to the 50th percentile are in the normal range for both sexes. The 75th percentile values can also be considered as normal, but are in the upper limit of normality, where the presence of a cardiovascular risk may be associated. In our study, glucose concentrations were not influenced by BMI and age.

According to Helene et al.[25] these parameters added to insulin and lipid profile evaluation can be used to monitor cardiovascular risk. Insulin values were positively correlated with BMI in both sexes. Rizzo et al.[26] showed in their study that adolescents with overweight, obesity and extreme obesity, had high average values of insulin, 13.27 ± 9.9 µIU/ml; 14.08 ± 7.64 µIU/ml and 16.13 ± 8.07 µIU/ml. If we compare the distribution of insulin values found in our sample group with those from Rizzo et al (2013), above the 75th percentile similar values were observed in overweight adolescents, while above the 90th percentile values are similar to those observed in extremely obese adolescents.

Cortisol is considered the stress hormone and the stress values can be influenced by several biological conditions, including increased body weight. The weight excess associated with insulin resistance and the metabolic syndrome in children and adolescents are sufficient to cause metabolic stress and an elevation of cortisol levels. In our study, we found no association between cortisol levels and BMI; however, in the study published by Reinehr and Andler[27], the weight loss of obese children and adolescents promoted a reduction in cortisol levels and insulin resistance. But some studies showed a large association between elevated serum cortisol and metabolic syndrome[3,28]. Cortisol concentration presented a tendency to increase with age for both sexes.

Due to our results, we suggest that in clinical practice, leptin may be considered a marker for the onset of puberty[29], and should be related to inflammatory parameters. A more complex assessment should be preferred to the determination of solely fasting glucose in order to prevent cardiovascular disease and diabetes. Thus, for adolescents above the percentile 75 for glucose (>than 95 mg/dl), a complementary evaluation, for assessment of global health status should be considered. Regarding insulin, adolescents with percentile equal to or higher than 75th percentile (>11.90 µIU/ml) should have a broader evaluation. Children with cortisol equal or above the 75th percentile (>15.0 µg/dl) should also receive a complementary assessment related to the factors involved in the metabolic syndrome and other precursors of stress. Smoothed centile curves could be used in clinical practice as possible reference values.

The HELENA study has several strengths. The sampling procedure and the strict standardization of the fieldwork among the countries involved in the study avoided to a great extent confounding bias due to inconsistent protocols and different laboratory methods,

which makes comparing results from isolated studies difficult. The main contribution of the present data is, for the fi Th time, to give a global overview of adolescent leptin, cortisol, insulin and glucose status in Europe. In the absence of reference values and specific cut-off points for this age group, percentile distributions as presented can be used in clinics and further research. The HELENA study performed in European adolescents aged 12.5 – 17.5 from ten different countries has given a unique opportunity to analyze hormonal concentrations in a large sample with a standardized methodology. The analysis of adiponectin was foreseen in the HELENA study, but unfortunately data were finally not available and could be considered a limitation in the context of the present study.

Conclusion

The reference curves established for leptin, cortisol, insulin and glucose, performed in the present study could be used in clinical practice and extrapolated to adolescents from other parts of the world, with similar ethnical background. In adolescents, not only BMI is associated with leptin, but also sex and age. . Depending on the aim of the study, current data can contribute to elucidate which parameter should be included in further studies. This paper could contribute to public health offering data in apparently healthy adolescents.

Disclosure

The content of this paper reflects only the author's view and the rest of HELENA Study members are not responsible for it. The writing group takes sole responsibility for the content of this article.

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Conflict of interests

None.

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