

CLINICAL STUDY

The relationship between leptin, gonadotropic hormones, and body composition during puberty in a Dutch children cohort

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Abstract

Objective: To investigate the relationship between leptin concentrations, gonadotropic hormone concentrations, and body composition during puberty in a Dutch children cohort.

Design: In a cohort of 98 children, we determined anthropometric measurements, body composition, and concentrations of leptin, FSH, and LH.

Results: Sex differences were observed from Tanner stage 1 onwards in weight, body fat percentage, and leptin/fat mass ratio. In boys and girls, the relationship between leptin concentrations and FM was weaker at Tanner stage 2 ($R^2=0.33$ and $R^2=0.39$; $P<0.001$), 3 ($R^2=0.27$ and $R^2=0.36$; $P<0.002$), and 4 ($R^2=0.21$ and $R^2=0.28$; $P<0.03$) than at Tanner stage 1 ($R^2=0.51$ and $R^2=0.67$; $P<0.001$) and 5 ($R^2=0.46$ and $R^2=0.78$; $P<0.01$). In girls, a peak in leptin concentrations (8.5 ± 6.0 ng/ml) preceded a peak in LH and FSH concentrations (15.1 ± 3.5 and 5.0 ± 4.5 IU/l). A lead/lag relationship was observed of leptin at Tanner stage 1 to LH and FSH at Tanner stage 2 ($R^2=0.12$, $P<0.05$ and $R^2=0.18$, $P<0.05$). In boys, there was no peak in leptin, LH, and FSH; additionally, leptin at Tanner stage 3 was related FSH at Tanner stage 4 ($R^2=0.17$, $P<0.04$).

Conclusion: In boys and girls during puberty, factors independent of fat mass become (transiently) more important in the regulation of plasma leptin concentrations. Moreover, in girls, leptin is suggested to act as a permissive factor for the onset of puberty, while, in boys, leptin has a different timing and possibly different function.

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Introduction

The achievement of a critical body weight (BW) or fat mass (FM) is thought to play a prominent role in the start of sexual maturation (1, 2), since underfeeding and malnutrition in humans, as seen in anorexia, is related to a delay in the onset puberty (1–3). Leptin has been proposed as a physiological link between adiposity status and the start of sexual maturation (1–5). Several cross-sectional studies and three small longitudinal studies support this theory since they have found that leptin concentrations rose prepubertally, but then decreased at the initiation of puberty, as gonadal hormone concentrations began to increase (1, 4, 6–11).

The proposed mechanism behind leptin as a trigger to puberty, beholds that leptin independently of FM acts on the hypothalamic LHRH pulse generator (12–14). Consequently, LHRH stimulates the release of LH and FSH from the pituitary, which in turn stimulate the gonads to release testosterone and estradiol (E_2 ; 12–14). Testosterone and E_2 inhibit the secretion of LHRH and respectively LH and FSH, which thereby form a negative feedback loop (13, 14). Moreover, testosterone inhibits leptin secretion from the adipocytes, which forms a

second negative feedback loop (14–16). A positive feedback loop is formed by E_2 , which stimulates leptin secretion from the adipocytes (14, 16). This proposed mechanism suggests that increased gonadotropic and gonadal hormones concentrations may alter the relationship between leptin concentrations and body fat during puberty.

Most studies, however, which investigated the role of leptin as a trigger to puberty, did not determine body composition (6), or were done in transversal cohorts (1, 6, 8, 9) and relatively small longitudinal cohorts (4, 10, 11). Consequently, previous studies have been unable to test the hypothesis that leptin and gonadotropic hormones are temporarily related during puberty, and whether consequently the relationship between leptin and body composition is temporarily altered. Therefore, the objective of our longitudinal study was to investigate the relationship between leptin concentrations, gonadotropic hormone concentrations, and body composition during puberty in a Dutch children cohort. Therefore, we determined in 98 children anthropometric measurements, body composition, and concentrations of leptin, FSH, LH, testosterone, and E_2 .

Subjects and methods

Subjects

Subjects were recruited from a Dutch Caucasian cohort of children born between 1990 and 1993 (17, 18). As infants, these children and their mothers participated in i) a study of essential fatty acids during pregnancy and pregnancy outcome (17), and ii) a study, performed between 1997 and 2000, about the long-term effects of fetal essential fatty acid availability (19). Anthropometric data were available from these children and no interventions were implemented. To evaluate the development of obesity and related parameters, follow-up studies were performed with 98 children, and the dropout rate was 0% (18).

Study design

Each child and one of his or her parents gave written informed consent to participate in the study, which was approved by the Central Committee Human Research and by the Medical Ethical Committee of the Maastricht University. At 1600 h after a 3-hour fast, children's age, BW, height, body mass index (BMI), body composition (7, 18), leptin concentrations, FSH concentrations, LH concentrations, testosterone concentrations, and E_2 concentrations were determined.

Measurements

Anthropometry The children's BW was determined using a digital balance accurate to 0.1 kg (Sauter D7470, Ebingen, Germany) and height was determined using a wall-mounted stadiometer (Seca, model 220, Hamburg, Germany). Measurements were executed in underwear and after voiding the bladder. BMI in childhood changes substantially with age and was calculated by $BW/height^2$ (kg/m^2), and to define normal, overweight, and obesity in children, we used the specific cut-off points described by Cole *et al.* 2000 (20). For example, at age 12, a BMI of 21.22 and 21.68 kg/m^2 in boys and girls is defined as overweight, and a BMI of 26.02 and 26.67 kg/m^2 as obese. The pubertal stage was documented in all children according to the classification by Tanner (21). The Tanner stage is defined based on physical measurements of external primary and secondary sex characteristics, namely development of breast and pubic hair in girls or development of genitalia and pubic hair in boys (21). At age 15, boys in Tanner stage 3 had testosterone levels of 8.3 ± 5.1 ng/l and boys with Tanner stage 5 had testosterone levels of 16.5 ± 5.4 ng/l. At age 15, girls in Tanner stage 3 had E_2 levels of 14.8 ± 15.8 and girls with Tanner stage 5 had E_2 levels of 32.7 ± 32.4 ng/l. Tanner stage definition was thus supported by plasma

testosterone and E_2 measurements (ng/l) at age 15. Anthropometric measurements were determined at ages 7, 12, 13, 14, and 15.

Body composition Body composition was measured using the deuterium dilution technique (D_2O). D_2O dilution was used to measure total body water (TBW). Subjects were asked to collect a urine sample in the evening just before drinking the deuterium-enriched water solution. After ingestion of this solution, no further consumption was allowed. Ten hours after drinking the water solution, another urine sample was collected. The dilution of the deuterium isotope is a measure of the TBW of the subject. Deuterium was measured in the urine samples with an isotope ratio mass spectrometer (VG-Isogas Aqua Sira, VG Isogas, Middlewich, Cheshire, England). TBW was obtained by dividing the measured deuterium dilution space by 1.04. Fat-free mass (FFM) was calculated by dividing TBW by the hydration factor 0.73 (22–24). FM was determined as $BW - FFM$. Body composition was measured at ages 12, 13, 14, and 15.

Leptin At 1600 h after a 3-hour fast, blood was drawn for determination of leptin concentrations. Plasma leptin concentrations were measured with a double-antibody, sandwich-type ELISA that used a MAB specific for human leptin. The lower limit of detection is 0.5 $\mu g/l$ and the upper limit is 50 $\mu g/l$. The intra- and interassay coefficient of variations (CVs) were 9 and 12% respectively. The leptin concentrations of normal-weight subjects range from 2 to 12 $\mu g/l$. Plasma leptin concentrations (ng/ml) were measured at ages 7, 12, 13, 14, and 15.

FSH At 1600 h after a 3-hour fast, blood was drawn for determination of FSH concentrations. Plasma FSH concentrations were measured with a double-antibody, sandwich-type ELISA that used a MAB specific for human FSH. The lower limit of detection is 0.100 IU/l and the upper limit is 200.0 IU/l. The intra- and inter-assay CVs were 1.8 and 5.1% respectively. The FSH concentrations range from 1.5 to 12.4 IU/l in boys and from 1.7 to 21.5 IU/l in girls. Plasma FSH concentrations (IU/l) were measured at ages 12, 13, 14, and 15.

LH At 1600 h after a 3-hour fast, blood was drawn for determination of LH concentrations. Plasma LH concentrations were measured with a double-antibody, sandwich-type ELISA that used a MAB specific for human LH. The lower limit of detection is 0.100 IU/l and the upper limit is 200 IU/l. The intra- and inter-assay CVs were 0.8 and 2.0%, respectively. The LH concentrations range from 1.7 to 8.6 IU/l in boys and from 1.0 to 95.6 IU/l in girls. Plasma LH concentrations (IU/l) were measured at ages 12, 13, 14, and 15.

Statistical analysis

Differences between two groups were determined using unpaired *t*-tests. Differences over time and between conditions were determined using two-factor ANOVA with repeated measures. Relationships between dependent and independent variables were determined using simple linear regression models. To investigate a time-dependent relationship in the sequence of the hormonal changes during puberty, we used the lead/lag technique, which correlates hormones secreted in one stage, with those secreted in the next stage (11). All tests were two-sided and differences were considered significant at $P < 0.05$. Values are expressed as mean \pm s.d.

Results

At Tanner stage 1, we collected data from 95 (57 m/38 f) children, at Tanner stage 2 from 95 children (54 m/41 f), at Tanner stage 3 from 95 children (55 m/40 f), at Tanner stage 4 from 63 children (35 m/28 f), as well as from 33 children (14 m/19 f) at Tanner stage 5. Eleven percent of the children were classified as overweight using specific cut-off points described by Cole *et al.* 2000 (20). In our cohort, annual height velocity in boys and girls was 5.7 ± 1.3 and 5.7 ± 1.2 cm/year at Tanner stage 1; 6.5 ± 1.8 and 5.7 ± 1.2 cm/year at Tanner stage 2; 6.9 ± 2.6 and 5.7 ± 2.4 cm/year at Tanner stage 3; 6.1 ± 3.0 and 3.1 ± 2.3 cm/year at Tanner stage 4; and 3.7 ± 3.1 and 1.5 ± 1.4 cm/year at Tanner stage 5. Figure 1 shows sex differences (boys versus girls) in BW at Tanner stages 1, 3, 4, and 5 (43.1 ± 12.5 vs 36.9 ± 14.2 kg, $P < 0.02$; 61.5 ± 10.5 vs 56.2 ± 9.7 kg, $P < 0.01$; 66.1 ± 9.5 vs 59.9 ± 9.7 kg, $P < 0.01$; 68.5 ± 9.2 vs 60.7 ± 8.8 kg, $P < 0.02$). Differences were absent at Tanner stage 2.

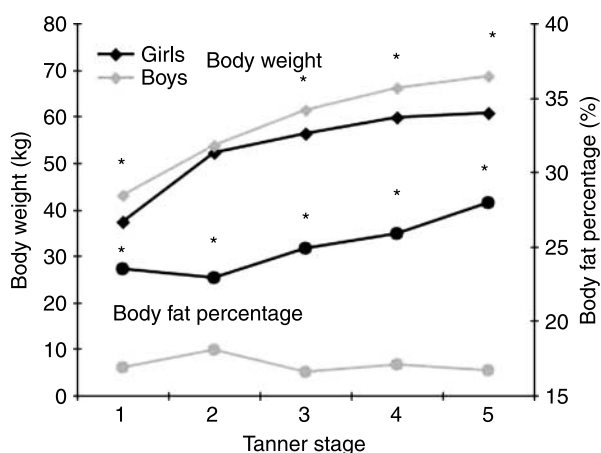


Figure 1 Mean of weight (kg) and body fat percentage (%) in the boys ($n=57$) and girls ($n=41$) as a function of Tanner stage. * $P < 0.05$ for differences between boys and girls (ANOVA repeated measures).

caused by a larger increase in BW in girls when compared with boys between Tanner stages 1 and 2 (15.6 ± 12.4 vs 12.4 ± 10.4 kg, $P < 0.18$), and a significant larger increase in BW in boys when compared with girls between Tanner stages 2 and 3 (6.6 ± 3.2 vs 3.9 ± 2.2 kg, $P < 0.001$). Additionally, sex differences in body fat percentage were shown at all Tanner stages (16.9 ± 7.2 vs $23.8 \pm 8.1\%$, $P < 0.05$; 18.1 ± 7.9 vs $22.9 \pm 5.9\%$, $P < 0.002$; 16.6 ± 6.7 vs $24.9 \pm 6.3\%$, $P < 0.001$; 17.1 ± 6.9 vs $25.9 \pm 5.7\%$, $P < 0.001$; 16.7 ± 5.9 vs $27.9 \pm 6.3\%$, $P < 0.001$). Because of sex differences, the results on body composition, leptin concentrations, and gonadotropic hormone concentrations were analyzed separately for boys and girls.

Figure 2(A) shows the mean of plasma leptin concentrations (ng/ml), LH concentrations (IU/l), and FSH concentrations (IU/l) in the boys ($n=57$) as a function of Tanner stage. In lean and overweight boys, no differences were found in the leptin and gonadotropic hormone patterns. The leptin concentrations increased from Tanner stage 1 to 2 (3.9 ± 2.3 – 4.9 ± 5.1 ng/ml, $P < 0.5$), and decreased significantly from Tanner stage 2 to 5 (4.9 ± 5.1 – 2.1 ± 1.9 ng/ml, $P < 0.01$). FSH concentrations increased from Tanner stage 1 to 4 (2.9 ± 2.3 – 3.6 ± 1.9 IU/l, $P < 0.09$), and LH concentrations significantly increased from Tanner stage 1 to 5 (1.8 ± 1.4 – 5.7 ± 2.8 IU/l, $P < 0.01$).

Figure 2(B) shows the mean of plasma leptin concentrations (ng/ml), LH concentrations (IU/l), and FSH concentrations (IU/l) in girls ($n=41$) as a function of Tanner stage. In lean and overweight girls, no differences were found in the leptin and gonadotropic hormone patterns. The leptin concentrations increased significantly from Tanner stage 1 to 2 (6.2 ± 4.9 – 8.5 ± 6.0 ng/ml, $P < 0.02$), decreased significantly from Tanner stage 2 to 4 (8.5 ± 6.0 – 6.2 ± 3.3 ng/ml, $P < 0.04$), and significantly increased again from Tanner stage 4 to 5 (6.2 ± 3.3 vs 10.4 ± 6.6 ng/ml, $P < 0.01$). The peak in leptin concentrations was followed by an increase in LH and FSH concentrations from Tanner stage 2 to 3 (3.4 ± 2.3 – 5.1 ± 3.5 IU/l, $P < 0.05$ and 5.1 ± 3.5 – 3.9 ± 4.3 IU/l, $P < 0.14$), and a decrease in LH and FSH concentrations from Tanner stage 3 to 4 (4.4 ± 5.0 – 5.0 ± 4.5 IU/l, $P < 0.24$ and 5.0 ± 4.5 – 3.8 ± 2.1 IU/l, $P < 0.33$).

Figure 3 shows the mean leptin concentrations per kg of FM of the boys ($n=57$) and girls ($n=41$) as a function of Tanner stage. Sex differences in leptin/FM ratio were observed at Tanner stages 1, 2, 3, 4, and 5 (0.49 ± 0.2 vs 0.70 ± 0.2 ng/ml per kg, $P < 0.009$; 0.51 ± 0.4 vs 0.73 ± 0.3 ng/ml per kg, $P < 0.005$; 0.38 ± 0.4 vs 0.55 ± 0.3 ng/ml per kg, $P < 0.04$; 0.26 ± 0.3 vs 0.39 ± 0.2 ng/ml per kg, $P < 0.04$; 0.17 ± 0.1 vs 0.56 ± 0.2 ng/ml per kg, $P < 0.001$).

Table 1 shows regression between plasma leptin concentrations (ng/ml) as the dependent variable and

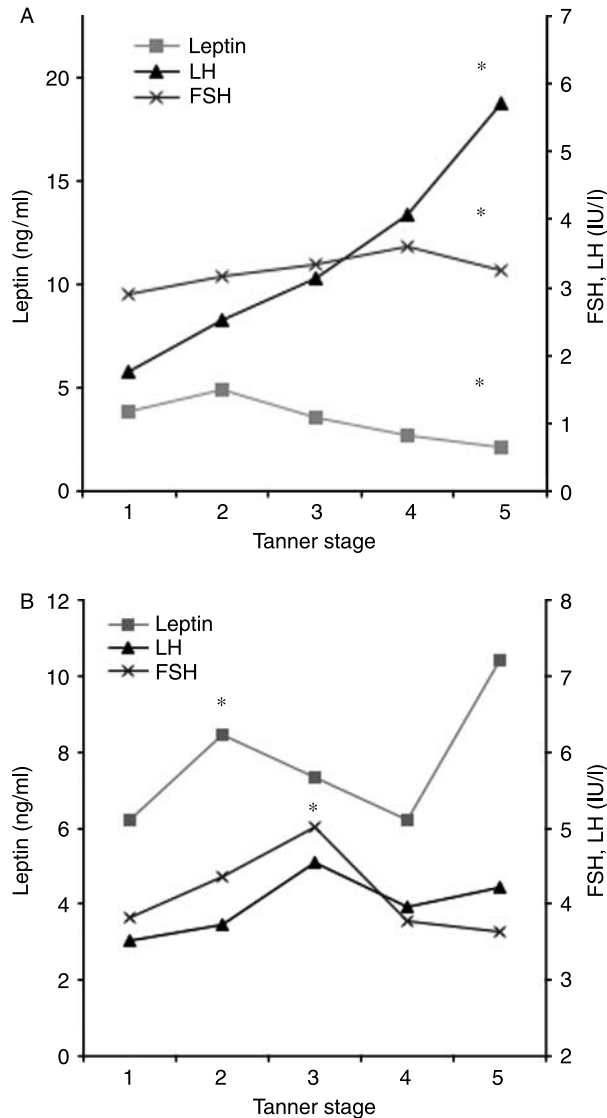


Figure 2 (A) Mean of plasma leptin concentrations (ng/ml), LH concentrations (IU/l), and FSH concentrations (IU/l) in the boys ($n=57$) as a function of Tanner stage. * $P<0.05$ for differences in time in the leptin and LH concentrations (ANOVA repeated measures). (B) Mean of plasma leptin concentrations (ng/ml), LH concentrations (IU/l), and FSH concentrations (IU/l) in the girls ($n=41$) as a function of Tanner stage. * $P<0.05$ for differences in time in the leptin and LH concentrations (ANOVA repeated measures).

FM (kg) as the independent variable in boys ($n=57$) and girls ($n=41$) at Tanner stage 1–5. In both boys and girls, a positive relationship was observed between leptin concentrations and FM from Tanner stage 1 onwards. Only at Tanner stages 2, 3, and 4, a smaller proportion of the variance in the leptin concentrations was accounted for by using FM as the independent variable.

To test whether leptin can be considered as the 'lead' hormone in relationship to LH, and FSH or vice versa, in

boys and girls leptin concentrations in a certain Tanner stage were paired with the other hormones in the following Tanner stage and vice versa, which results in 32 sets of paired data per child. In boys at Tanner stage 1, LH and FSH concentrations were related to Tanner stage 2 leptin concentrations ($R^2=0.16$, $P<0.02$ and $R^2=0.13$, $P<0.03$), in turn Tanner stage 3 leptin concentrations were related to Tanner stage 4 FSH concentrations ($R^2=0.17$, $P<0.04$).

In girls at Tanner stage 1, leptin concentrations were related to Tanner stage 2 LH and FSH concentrations ($R^2=0.12$, $P<0.05$ and $R^2=0.18$, $P<0.05$).

Discussion

The objective of our longitudinal study was to investigate the relationship between leptin concentrations, gonadotropic hormone concentrations, and body composition during puberty in a Dutch children cohort. We observed sex differences from Tanner stage 1 onwards in BW, body composition, and leptin concentrations per kg FM. This was in concordance with previous literature on developmental changes in anthropometry and leptin concentrations normalized to FM during puberty (8, 25).

Sex differences were also observed in the development of leptin, LH, and FSH concentrations over time. In girls, a peak in leptin concentrations was observed at Tanner stage 2, followed by a peak in LH and FSH concentrations at Tanner stage 3, thereby confirming results from previous cross-sectional and small longitudinal studies (1, 4, 6–11). In boys, no peak in leptin was observed at Tanner stage 2, as leptin decreased from Tanner stage 2 onwards and LH and FSH concentrations increased from Tanner stage 1 to 4, thereby

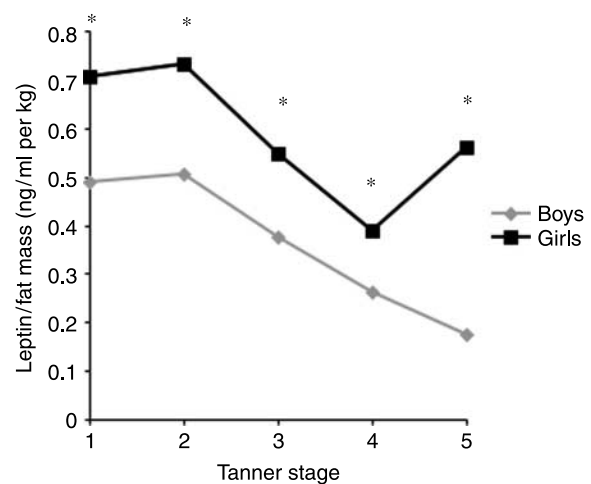


Figure 3 Mean leptin concentrations (ng/ml) per kilogram of fat mass of the boys ($n=57$) and girls ($n=41$) as a function of Tanner stage. * $P<0.05$ for differences between boys and girls (ANOVA repeated measures).

Table 1 Regression between plasma leptin concentrations (ng/ml) as the dependent variable and fat mass (kg) as the independent variable in boys ($n=57$) and girls ($n=41$) at Tanner stage 1–5.

Tanner stage	R^2	P value
Boys		
1	0.51	0.001
2	0.33	0.001
3	0.27	0.001
4	0.21	0.01
5	0.46	0.01
Girls		
1	0.67	0.001
2	0.39	0.001
3	0.36	0.002
4	0.28	0.03
5	0.78	0.001

confirming results from previous small longitudinal studies (4, 26). Transversal studies, however, did show a leptin peak in boys during puberty (1, 6), which may be explained by methodological differences as well as presentation of the data by age group instead of by pubertal stage.

The observations of our longitudinal study shed more light on the temporal alterations in the relationship between leptin and body composition (8). Both in boys and girls, the leptin/FM ratio decreased from Tanner stage 2 onwards. In boys, this decrease continued throughout puberty, while, in girls, this ratio increased again at Tanner stage 5, which confirms results by Horlick *et al.* (8). These results imply that during puberty factors independent of FM become (transiently) more important in the regulation of plasma leptin concentrations.

To investigate a time-dependent relationship in the sequence of the hormonal changes during puberty, we used the lead/lag technique, which correlates hormones secreted in one stage, with those secreted in the next stage (11). In girls, we observed that prepubertal leptin concentrations (Tanner stage 1), when acting as a lead, related to early pubertal LH and FSH concentrations (Tanner stage 2), as observed in both boys and girls by Masgood *et al.* (11). These results imply a temporal relationship between leptin and gonadotropic hormones during early puberty.

In boys, opposing results were found: prepubertal LH and FSH concentrations (Tanner stage 1) were related to early pubertal leptin concentrations (Tanner stage 2), as observed in boys and girls by Masgood *et al.* (11). They, however, observed prepubertal leptin concentrations to be related to early pubertal gonadotropic hormones and vice versa in both boys and girls (11), while we observed the first relationship in girls and the second one in boys. The discrepancy between the two studies is presumably caused by the difference in study population, because we analyzed boys and girls separately, each group consisting of ~40 children,

and Masgood *et al.* (11) analyzed only 13 boys and 7 girls together as one group, thereby being unable to separate the two different relationships. It should be noted that in both studies, LH and FSH concentrations were determined in morning urine or plasma samples. Both methods do not provide information on the night-day rhythm and on pulsatile secretory patterns, typical for the gonadotropic hormones (27). Moreover, we observed in boys a lead/lag relationship of leptin concentrations at Tanner stage 3 and FSH concentrations at Tanner stage 4. Our results show that during puberty the relationship between leptin and gonadotropic hormones is sex specific, since during puberty in boys leptin seems to have a different function and different timing. Still, previous studies have shown that leptin is essential in reproductive functioning in both boys and girls, as male and female sterile ob/ob mice became fertile again by leptin treatment (28). The observations from our and other studies show that a deficiency in leptin and FM as seen in anorexia nervosa or hyperleptinemia as seen in morbid obesity will disturb the role of leptin during puberty, and thereby the start and progression of puberty (3).

Thus, we observed in our longitudinal cohort that during puberty, the leptin/FM ratio decreased from Tanner stage 2 onwards. In girls, a peak in plasma leptin concentrations precedes a peak in LH and FSH concentrations, which supports a permissive role for leptin in the onset of puberty in girls. In girls, temporal relationships were observed between leptin and gonadotropic hormones during early puberty. In boys, however, there was no peak in leptin, LH, and FSH, and leptin was only related to LH and FSH during late puberty. We therefore conclude that in boys and girls during puberty, factors independent of FM become (transiently) more important in the regulation of plasma leptin concentrations. Moreover, in girls, leptin is suggested to act as a permissive factor for the onset of puberty, while, in boys, leptin has a different timing and possibly different function.

Declaration of interest

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

F R, S V, S L, and N V carried out the study, collected and analyzed the data, and F R wrote the largest part of the manuscript. M W and A N supervised F R, S V, S L, and N V. Planning, processing the results, and writing the manuscript were done under general supervision by M W and A N.

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