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Time course and determinants of leptin decline during weight loss in obese boys and girls

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Abstract

Objective. To investigate whether changes in leptin concentrations during weight loss can be explained by gender, puberty, baseline adiposity and changes in adiposity, body composition, rate of weight loss, physical activity and insulin concentrations. *Design*. A longitudinal study with 9 repeated measures during a 12-week weight loss programme. *Subjects*. Fifty-three boys and 62 girls (7.9–15.2 years) with body mass index (BMI) standard deviation scores (SDS) of median 2.78 and 2.70, respectively. *Measurements*. Height, weight, fat mass percentage assessed by bioimpedance, Tanner stages, testicular size, physical activity scores, blood leptin (ng/ml) and insulin concentrations (pmol/l) were measured at baseline, and except for Tanner stage and testicular size, repeated regularly during the programme. *Results*. The weight loss was accompanied by a steep decline in leptin concentrations during the first 10–11 days, followed by a less steep decline until day 82. Leptin declined to 39% in boys and 51% in girls of the level that was expected given the relationship at baseline between leptin and BMI SDS, and the BMI SDS changes during weight loss. The biphasic leptin decline was independent of gender, puberty, baseline adiposity or concomitant changes in BMI SDS, fat mass percentage, rate of weight loss, physical activity scores or insulin concentrations. *Conclusion*. The biphasic leptin decline, which exceeded the level expected, was independent of puberty, baseline adiposity and changes in adiposity, body composition, rate of weight loss, physical activity scores and insulin concentrations. The dissociation of the leptin-weight relationship during weight loss may contribute to the general leptin variability in obese subjects.

Key words: *BMI SDS, body composition, child, insulin, leptin, longitudinal study, obesity, puberty, weight loss*

Introduction

Evidence is accumulating that leptin is an important signal in human energy regulation (1), since the absence of leptin results in severe early-onset obesity, which can be reversed by leptin administration (2), and low-dosage leptin reverses skeletal muscle, autonomic and neuroendocrine adaptations to maintenance of reduced weight in humans (3). However, there is a considerable unexplained variability in leptin levels in individuals with comparable fat masses (4), which complicates understanding leptin's role as a potential feed-back signal in body weight regulation.

Leptin is secreted in a diurnal pattern (5), and changes in circulating concentrations have been found to be associated with changes in fat mass (6), gender and pubertal stages (7), adipose cell size and localisation (8,9), menstrual cycle (10), levels of insulin (11), cortisol, growth hormone (12), testosterone (13), 24-hour fasting, over-eating and exercise (14–16).

Generally, leptin can be seen as reflecting the amount of energy stored in the weight stable individual (17), as well as being a sensor of changes in energy balance (14–16). Although loss of fat mass is accompanied by a decrease in leptin, e.g. a 10%

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loss in fat mass has been associated with a 53% reduction in leptin levels in adults on a weight loss programme (4), it is uncertain whether it is the diminishing fat mass *per se* and/or the negative energy balance that explains the changes in leptin seen during weight loss (14–16). Further, results regarding leptin in childhood obesity are scarce and earlier studies have measured leptin either cross-sectionally (6,7,17–22) or only a few times, typically limited to before and after weight loss, providing little information about the time course during weight loss (23–27).

We have conducted a detailed longitudinal study of the leptin alterations in a controlled in-house environment offering stable negative energy balance in children. We investigated the changes in leptin and how they were related to gender and puberty, and to changes in adiposity, body composition, weight loss rate, physical activity and insulin concentrations.

Methods

Design

Groups of obese children of school age were examined on Day 1, 4, 8, 14, 23, 33, 48, 66 and 82, with minor variation owing to examination times falling at weekends or holidays etc. The examinations included interviews and assessment of Tanner stages (Day 1 and 82), weight, height, bioimpedance and venous blood samples for leptin and insulin (Day 1, 14, 33 and 82), and weight, height, bioimpedance and ear capillary blood samples for leptin and insulin (Day 4, 8, 14, 23, 48 and 66). Blood sampling was successful in 97% of all attempts. The ethical committee of the County of Copenhagen approved the study. Written informed consent was obtained from the parents of the participating children.

Setting

The institution "Julemærkehjemmet", Skælskør, Denmark offered a weight reduction programme consisting of restricted low-fat diets with a fixed level of energy intake at 6500–7000 kJ per day and an abundance of mandatory, as well as, optional physical activity. Physical activity was scored daily using a standardised method by the local staff and scores were summed weekly. The children stayed at the institution for approximately 12 weeks and were allowed home-visits during Easter, summer, autumn, Christmas and winter vacations, as well as, every third weekend.

Children

The children were referred to the weight loss programme by a Chief Paediatrician and were without any recognised associated medical conditions. During the study period from May 1997 to January 1999, 232 children were identified as eligible to participate, with 120 agreeing to do so.

Procedures

Height was measured by stadiometer to the nearest 5 mm. Weight was measured to the nearest 0.1 kg on a SECA Delta Scale (Model 707, Simonsen & Weel, Denmark) with participants wearing only undergarments. Body mass index standardised scores (BMI SDS) were calculated by the LMS method based on growth data generated in Danish boys and girls (28). The BMI SDS expresses the standard deviation from the mean BMI in the age and gender adjusted population. The rate of weight loss, Δweight (kilograms)/Δtime (days), was calculated from visit to visit, throughout the study period. Physical activity scores were scored daily for each child and the scores were summed up weekly. Physical activity scores were defined according to the degree of energy required and time spent at that particular activity (running, swimming, walking, bicycling, playing) recorded by a local empirically developed scheme. Energy intake was not measured individually, but the diet was within 6500–7000 kJ in each child per day. Ingestion of all meals, as well as portion-size and components of the meal, was supervised. Binge eating was avoided.

Fat-free mass percentages were obtained by the bioelectric multi-frequency impedance analysis using an SFB3 impedance instrument (Impedimed – Uniquest Ltd. Australia) and calculated according to the equation proposed by Schafer et al. (29), which has been validated against total body potassium (29). We also applied the equation of Wabitsch et al. (30), validated by deuterium dilution (30), and this equation gave essentially similar results. Puberty stage was rated according to Tanner stages after evaluation of pubic hair and breast and testicular size, where the latter was assessed by Prader's orchidometer. The same medical doctor (Jens-Christian Holm) and the same technician (Oda Troest) carried out all examinations.

Blood samples were collected from each child between 7.00 am and 8.30 am after an overnight fast during the weight loss programme. The serum was frozen at -20°C until analysis. Radio-immunoassays for leptin were measured in duplicate using the Linco Human Leptin RIA Kit (Linco Research Co, St Louis, MO, USA). The inter-assay coefficient

of variation was 4.0% and the intra-assay coefficient of variation was 7.6%. A limit of agreement analysis (Bland-Altman, 1986) revealed no differences between leptin measurements obtained by simultaneous venous and ear capillary sampling ($p = 0.55$), which allowed us to use leptin levels obtained by either method. The DAKO Insulin ELISA (DAKO Diagnostic Ltd., United Kingdom) was used and it showed an inter-assay coefficient of variation of 7.5% and an intra-assay coefficient of variation of 6.5%.

Statistical methods

Leptin was log-transformed in order to achieve approximate normality, variance homogeneity and linearity.

In order to compare our findings with published cross-sectional data and to estimate expected leptin levels, a simple linear regression of leptin against BMI SDS was performed on data at entry. Expected leptin concentrations during the weight loss programme were then estimated from the relationship between leptin and BMI SDS at baseline, and the BMI SDS changes observed at a given point in time during weight loss; these expected leptin values were compared with observed leptin levels.

Correlation coefficients (r) between leptin and BMI SDS at each examination were estimated and partial correlation coefficients were estimated after adjustment for puberty (and body composition).

In order to estimate leptin variability, and to explain patterns of leptin development during weight loss while taking advantage of the repetitive sampling in each child, a parametric multivariate linear regression model for longitudinal data was applied (31). Those children with three or fewer examinations were excluded from the longitudinal analysis. The shape of the leptin variogram (not shown) encouraged a model that contained three elements of variation. These elements were measurement error, a random effect accounting for inter-individual variation and an exponential serial correlation. In the mean value structure, BMI SDS and a variable describing pubertal development at Day 1 was included as independent variables. Whether or not menarche had occurred was used in the analysis of girls and log-transformed testicular size was used in the boys. An index termed rate of weight loss was calculated by Δweight (kg)/Δ time (days). In the mean value structure, the log-transformed BMI SDS, the log-transformed testicular size or menarche and the rate of weight loss were used as independent variables.

Finally, time was included, expressed as examination number or as continuous, as time since start of the weight loss programme. The leptin decline was non-linear and appeared in two phases. A model with a stepwise linear dependence between leptin and time, measured in days, was applied in order to model the change of leptin over time. In order to estimate the time point that separates the phases of the time-dependent changes in leptin, a maximum likelihood criterion was applied, which revealed the optimal point of separation in both boys and girls.

Possible selection bias due to loss of subjects was evaluated by testing whether subjects dropping out differed from those completing the study with respect to baseline parameters.

Results

Of the 120 children included in the weight loss programme, 90 children completed the planned investigative program; 25 were excluded, because they refused intravenous sampling, whereas 5 were excluded due to being of normal weight. Table I shows the characteristics of the children at baseline and at day 82. The age, baseline weight and weight changes during the weight loss programme were similar in boys and girls. At each examination, the number of children varied due to illness or late returns after weekends.

There was no significant difference in age between those completing and those dropping out of the programme ($p = 0.59$), but both boys and girls dropping out were heavier: the mean BMI SDS at baseline of those who completed the programme was 2.62 ± 0.57 compared with a mean BMI SDS of 2.91 ± 0.60 in those who dropped out ($p = 0.023$). Owing to the availability of a more complete dataset, we have used BMI SDS rather than fat mass percentage in the analysis, but all established relationships were re-run using fat mass percentage, and results were essentially the same.

Table II shows the development of median BMI SDS and concurrent median leptin levels during the weight loss programme. Both baseline median BMI SDS and the BMI SDS decline during weight loss were similar in boys and girls. BMI SDS was reduced by 0.93 SD in boys and by 0.89 SD in girls. Leptin levels were higher in girls, than boys. At baseline, there was an inverse relationship between leptin and testicular size with the regression coefficient $ß = -0.143 \pm 0.064$ ($p = 0.0032$). Median leptin levels decreased in boys by 16.9 ng/ml and in girls by 18.6 ng/ml. The correlation between BMI SDS and leptin levels was stronger in girls than in boys.

Table III shows the regression coefficients expressing the relationship between leptin and BMI SDS through all examinations in boys and girls. The regression coefficients were adjusted for menarche

Table I. Characteristics of children at baseline and after weight loss in boys and girls.

	Boys				Girls			
Baseline	N	P 10#	Median	P 90#	N	P 10#	Median	P 90#
Age (yr)	52	10.6	12.1	13.2	58	10.8	12.1	13.5
Weight (kg)	52	51.7	63.2	80.9	58	50.2	67.8	84.0
Height (m)	52	1.48	1.60	1.67	58	1.44	1.59	1.68
BMI SDS*	52	2.22	2.78	3.62	58	1.90	2.71	3.28
Body Fat%	39	31.8	41.8	49.0	39	33.4	44.5	49.3
Fat mass (kg)	39	15.1	25.0	38.0	39	17.8	28.9	39.7
Leptin (ng/ml)	47	10.1	21.1	32.6	51	17.5	27.8	42.6
Insulin (pmol/l)	45	36	61	95	43	47	92	190
Tanner Breast					56	1	2	4
Tanner Pubertal	51	1	2	5	56	1	2	4
Tanner Gonadal	51	1	2	4				
Testis Right (ml)	51	1	3	10				
Testis Left (ml)	50	1	3.5	9				
Menarche (yr)					21	11	12	13
Day 82	Boys				Girls			
	N	P 10#	Median	P 90#	N	P 10#	Median	P 90#
Age (yr)	41	10.6	12.4	13.5	46	10.7	12.2	13.9
Weight (kg)	41	45.9	56.3	67.2	45	43.3	61.7	72.8
Height (m)	41	1.49	1.62	1.69	45	1.46	1.60	1.69
BMI SDS*	41	1.11	1.84	2.76	45	1.18	1.84	2.66
Body Fat%	33	30.6	34.8	38.7	31	30.7	38.8	40.9
Fat mass (kg)	33	14.7	18.9	25.2	31	12.4	22.9	29.1
Leptin (ng/ml)	41	3.0	4.4	8.0	46	4.8	8.8	18.8
Insulin (pmol/l)	35	24	31	52	36	36	48	78
Tanner Breast					35	1	2	4
Tanner Pubertal	35	1	2	5	35	1	2	4
Tanner Gonadal	35	1	2	5				
Testis Right (ml)	35	2	4	15				
Testis Left (ml)	35	1	4	15				
Menarche (yr)					19	11	12	13

*Body mass index (BMI) standard deviation scores (SDS).
#10% percentile (P10) and 90% percentile (P90).

status in girls and testicular size in boys at baseline. The regression coefficients were comparable in boys and girls during the weight loss programme.

Stratification of children by baseline BMI SDS quartiles showed that leptin declines were similar in boys (Figure 1A) and girls (Figure 1B) in all weight quartiles, even though leptin levels were higher in girls. The regression lines at baseline and at the last examination were parallel, illustrating a stable relationship between leptin and BMI SDS during weight loss in both genders. Figure 1 also shows that leptin levels had a greater reduction during weight loss than would have been expected from the baseline BMI SDS and the BMI SDS changes found during weight loss.

After adjustment for concurrent BMI SDS and pubertal development at baseline, the decline in leptin levels was steeper in the early phase, than in later phases of weight loss (Figure 2). The decline could be divided into two distinct phases and the time of transition between the two phases was estimated. Significant changes in the rate of the leptin decline took place at Day 10 in boys and at Day 11 in girls. In the first phase, leptin levels declined in boys to 53% and in girls to 60% (both $p < 0.0001$) of the values expected given the relationship between leptin, BMI SDS and puberty at entry, and BMI SDS changes seen during weight loss. In the remaining weight loss period, the leptin changes were less and leptin declined in boys to 39% ($p = 0.001$) and in girls to 51% ($p = 0.014$) of the expected leptin levels as determined by the relationship between leptin, BMI SDS and puberty at baseline, and the BMI SDS changes during weight loss. The regression coefficients (β) and corresponding standard deviations for leptin and time in the first phase were -0.060 ± 0.006 in boys and -0.045 ± 0.004 in girls, and during the remaining weight loss programme they were -0.0043 ± 0.0012 in boys and -0.0022 ± 0.0009 in girls. The leptin declines

Table II. Median values of leptin concentrations and Body mass index (BMI) standard deviation scores (SDS) with 5 and 95% percentiles during weight loss in boys and girls. Partial correlations (r) were statistically stronger and more stable among girls, than in boys.

	Days								
	1	4	8	14	23	33	48	66	82
Boys									
N	47	49	46	40	41	40	39	40	37
BMI SDS[#]	2.8	2.8	2.6	2.6	2.5	2.4	2.2	2.0	1.8
Percentiles									
5%	1.7	1.7	1.6	1.3	1.4	1.5	1.1	0.8	0.7
95%	3.6	3.6	3.6	3.6	3.5	3.4	3.3	3.0	2.9
Leptin									
(ng/ml)	21.1	15.1	11.6	9.6	8.5	7.2	5.9	5.0	4.2
Percentiles									
5%	8.7	4.1	4.7	3.6	3.6	2.7	2.3	2.4	2.4
95%	34.8	29. 7	24.9	19.7	24.8	13.4	15.6	11.9	11.3
Partial r	0.56	0.49	0.45	0.48	0.40	0.40	0.32	0.19	0.46
P	<0.0001	0.0004	0.002	0.002	0.01	0.01	0.05	0.25	0.005
Girls									
N	51	55	52	49	48	45	45	45	41
BMI SDS[#]	2.7	2.6	2.6	2.5	2.4	2.3	2.2	2.0	1.8
Percentiles									
5%	1.8	1.5	1.4	1.3	1.3	1.4	0.9	0.4	0.7
95%	3.3	3.3	3.2	3.2	3.1	3.1	3.0	2.9	2.8
Leptin									
(ng/ml)	27.8	20.4	18.5	14.0	15.4	13.2	11.8	9.3	9.2
Percentiles									
5%	15.5	8.6	8.4	5.1	6.3	5.1	4.7	4.4	4.5
95%	58.4	48.4	43.1	32.6	34.4	31.8	23.8	23.7	22.6
Partial r	0.56	0.59	0,63	0.63	0.62	0.65	0.66	0.47	0.47
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.002	0.003

[#]Body mass index (BMI) standard deviation scores (SDS).
Partial correlation inclines adjustment for puberty.

in the two phases were significantly different from zero, as well as different from each other (both $p < 0.001$).

Table III. Regression coefficients with corresponding standard errors show a stable relationship between leptin and body mass index (BMI) standard deviation scores (SDS) during weight loss in boys and in girls.

Day	Regression coefficients and standard errors Boys	Regression coefficients and standard errors Girls
1	0.48 ± 0.11	0.46 ± 0.10
3	0.53 ± 0.10	0.61 ± 0.09
7	0.39 ± 0.10	0.61 ± 0.09
14	0.40 ± 0.10	0.57 ± 0.09
23	0.40 ± 0.10	0.66 ± 0.09
32	0.46 ± 0.10	0.61 ± 0.09
47	0.38 ± 0.11	0.53 ± 0.08
65	0.18 ± 0.11	0.42 ± 0.08
82	0.29 ± 0.11	0.38 ± 0.09

Regression coefficients were generated by a longitudinal multivariate linear regression model and adjusted for testicular size in boys and menarche in girls. All relationships were significant with $p < 0.001$.

Overall, the rate of weight loss index was associated with leptin levels in boys ($p < 0.0001$) and girls ($p = 0.0004$). However, the rate of weight loss index showed a significant relationship with leptin levels only after 18 days of weight loss. Thus, the regression coefficients (β) between leptin and rate of weight loss in the first 18 days were 0.01 and 0.04 (both $p > 0.05$) in boys and girls, respectively, and in the rest of the weight loss period, 0.94 and 0.66 (both $p < 0.001$) in boys and girls, respectively.

The weekly physical activity scores of mandatory and optional physical activity were not associated with leptin changes in boys ($p = 0.18$) or girls ($p = 0.67$), and had no significant impact on the leptin decline or the day of transition of the leptin decline (data not shown).

Insulin levels were significantly associated with leptin levels, with the regression coefficient in boys being $\beta = 0.24$ ($p < 0.0001$) and in girls, $\beta = 0.10$ ($p = 0.002$) during the weight loss programme.

In spite of their own inherent relationship to leptin levels, adjustment for fat-mass percent, rate of weight loss and insulin levels did not contribute to

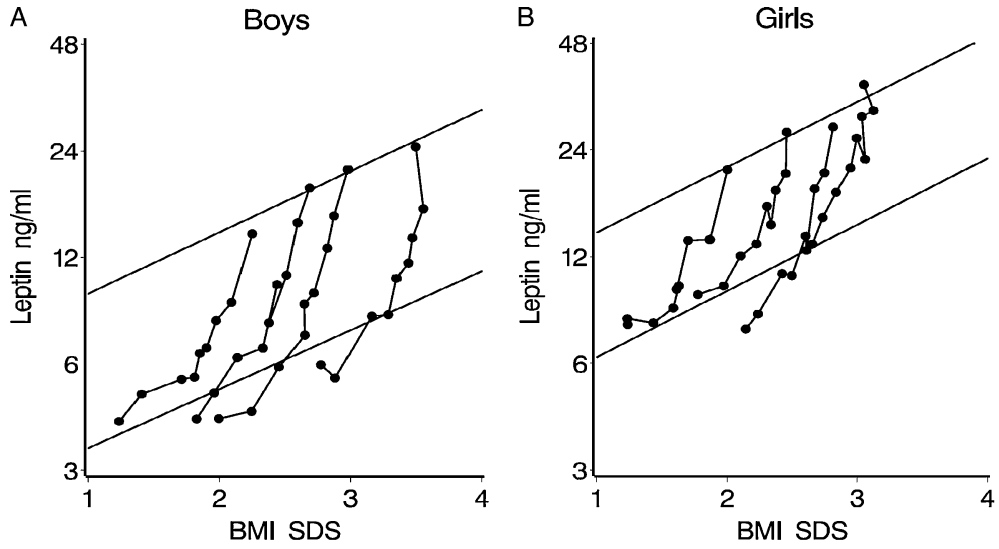

Figure 1. Leptin concentrations during weight loss stratified by body mass index (BMI) standard deviation scores (SDS) quartiles at baseline in boys (Figure 1A) and in girls (Figure 1B). Parallel regression lines at baseline (top) and at last examination (bottom) express a stable relationship between leptin and BMI SDS in both boys and girls. Leptin concentrations decreased similarly in all weight quartiles and in both genders.

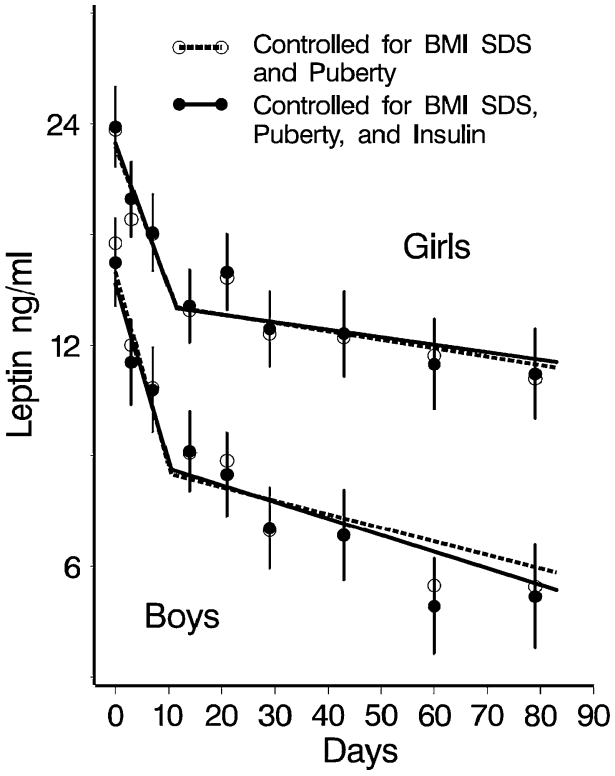

Figure 2. The decline in leptin concentrations during weight loss adjusted for gender, puberty and body mass index (BMI) standard deviation scores (SDS) (○), and after further correction for insulin concentrations (●). (Correction for fat mass and rate of weight loss are not shown but results are similar). Adjacent confidence interval bars illustrate that neither the shape, nor the degree of the leptin decline was explained significantly after adjustment for fat mass, rate of weight loss or insulin concentrations. The leptin decline was calculated according to a likelihood criterion applied in a longitudinal multivariate linear regression model.

the explanation of the excessive leptin decline or the day of transition of the rate of leptin decline (Figure 2).

Discussion

The novel findings of this longitudinal study are that leptin declined in a biphasic pattern evident after 10–11 days of weight loss in obese boys and girls, and that leptin declined beyond the expected level given the relationship between leptin and BMI SDS at baseline, and BMI SDS changes observed during weight loss. This suggests a functional dissociation of leptin and fat mass. The biphasic leptin decline and the greater than expected leptin decline could not be attributed to baseline adiposity, puberty or to changes in adiposity, body composition, rate of weight loss, physical activity scores or insulin levels. The dissociation of the leptin-weight relationship appeared even though the relationship at any given point in time during the weight loss programme was stable during the entire period.

In the present study, we sought to avoid the influence of diurnal leptin variation (5) by sampling fasting leptin levels early in the morning. Leptin declines were essentially uniform irrespective of body weight, as boys and girls with different weight quartiles at entry exhibited similar leptin declines during weight loss. This suggests that the decline in leptin was independent of baseline adiposity.

The biphasic leptin decline and the dissociation of leptin from weight may not have been observed in previous studies, since the leptin decline changed rate after only 10 days of weight loss and the leptin

decline seemed to be fat-mass independent. Furthermore, in the present study, indices of weight at any given point in time during the weight loss course tended to maintain the same relative relationship to leptin, as seen from the stable regression coefficients in both boys and girls, which suggests that the dissociation related to the general level of leptin and not to its inherent association with fatness, an aspect not addressed in earlier studies.

An important consideration is the completeness of the data. Children dropping out were heavier, but not different in terms of baseline age, and subsequent re-analysis without those dropping out during the programme gave similar results.

Leptin levels were higher in girls, and in boys, leptin had a significant inverse relationship with testicular size, which is in agreement with earlier reports (6,7,17,21). There exists conflicting evidence as to whether body composition differences explain the sexual dimorphism seen in leptin levels (22) or not (18,21). Sexual dimorphism may have implications in the leptin-weight relationship in the present study, as girls exhibited both strong partial correlation and regression coefficients between leptin and BMI SDS during weight loss, whereas partial correlations were weaker during weight loss in boys (also after adjustment for body composition, data not shown). This observation is the opposite of the findings by Reiterer et al. (23), who found that the correlations between percentage of fat mass and leptin increased after 3-weeks of weight loss in pubertal boys. However, the study of Reiterer et al. (23) and the present study differed in classification of puberty (Tanner stages vs. testicular size and menarche), number of children (62 vs. 120) and time-course studied (3 weeks vs. 12 weeks), which makes direct comparisons difficult.

Argente et al. (19) reported no correlation between leptin and BMI SDS in 14 obese children before and after 25% and 50% reduction of initial BMI SDS. Di Stefano et al. (25), studying 418 children with four measurements over two years, despite a considerable drop out, found significant partial correlations between leptin and $\triangle$BMI z-scores, when boys and girls were pooled together and gender subsequently adjusted for; this assumes the same effects in boys and girls and makes a comparison with our study difficult. Falorni et al. (20) reported a cross-sectional study of 390 obese and 320 normal weight subjects, and found a correlation between BMI SDS and leptin, except in normal weight girls, Tanner stage II–III. Overall, these findings show that relationships between leptin, baseline adiposity and changes in adiposity, body composition and puberty are still unclear.

Numerous studies have shown that leptin is reduced during weight loss (4,32). Considine et al. (4) and Wing et al. (32) showed that a 10% reduction in body weight was associated with leptin levels declining to 53% and 29% of initial levels, respectively. However, a study with 5 measurements during steady negative energy balance resulting in weight loss was associated with a leptin decline in women until week 5, after which leptin levels increased at 10, 20 and 40 weeks of continuous weight loss in low calorie dieters (33), suggesting that the diminishing fat mass and concurrent negative energy balance in the long term may not be associated with declining leptin levels. Reinehr et al. (27) measured leptin before and after one year of significant weight loss in 11 children and did not find a significant leptin decline. Since our study was concluded at week 12, we do not know if a later rise in leptin levels takes place in the children who are able to continue weight loss.

In the present study, we adjusted for baseline adiposity, change in adiposity, rate of weight loss and physical activity points. However, these factors did not explain the leptin decline or the day of transition of the leptin decline, even though rate of weight loss exhibited a significant relationship with leptin, raising the question as to whether leptin does reflect energy balance (14–16) or whether leptin reflects something else. A limitation of the present study is that our measure of physical activity is not validated, instead we used rate of weight loss as a proxy for the resultant negative energy balance in a specified period. More precise estimates of changes of energy balance components will be needed to further understand the mechanisms by which leptin alterations are involved during weight changes. However, precise components of energy balance are difficult to measure besides those obtained when registering weight changes in subjects out of metabolic wards.

A close relationship has been established previously between fasting leptin and insulin (34) and their changes during weight loss, fasting, and overeating (11,35,36). Chronic, but not acute insulin supplementation stimulates the secretion of leptin (11) and both hormones are secreted in proportion to fat mass (4,37) and are active centrally by reducing appetite inducing weight loss (38,39). Insulin and leptin showed significant relationships in the children during weight loss, but changes in insulin did not explain the observed leptin decline or the day of the transition of the leptin decline.

A dissociated relationship between leptin and fat mass has been described previously in pregnant women, where leptin levels rose in the third trimester out of proportion with the fat depots in the women (40). After delivery, leptin levels fell immediately in

the first hours post partum aligning with the relationship between leptin and fat mass seen before pregnancy (41). In both puberty and pregnancy, leptin resistance has been proposed to have a physiological implication by allowing the build-up of fat mass crucial to subsequent reproductive function, including support of the development of the foetus (6).

In rodent models, as a response to fasting, decline in leptin levels stimulates anabolic pathways (and reduces catabolic activity) (42), resulting in increased appetite and weight gain (43). In human subjects maintained on a 10% reduced weight, leptin administration also reverses weight-loss induced energy-sparing adaptations (3). The steep biphasic leptin decline and the dissociated leptin weight relationship suggest the hypothesis (44) that under energy depletion, a regulatory stimulus initiates anabolic signalling resulting in the weight rebound most often seen after weight loss. In an evolutionary perspective, this may have preserved life (42). Our finding of an early biphasic leptin decline after initiating weight loss, combined with the specific dissociation of general leptin level and body weight, may have important implications for weight loss regimens and for the attempts to prevent weight regain. These findings suggest that early supplementation with leptin pathway downstream analogues may be worth investigating.

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