Tracking of Leptin, Soluble Leptin Receptor, and the Free Leptin Index during Weight Loss and Regain in Children

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Keywords
Child · Free leptin index · Leptin · Longitudinal study · Obesity · Puberty · Soluble leptin receptor · Weight loss · Weight regain

Summary
Objective: To investigate changes in leptin and soluble leptin receptor (SLR) concentrations, and in the free leptin index (FLI) during weight loss and subsequent weight regain; and to ascertain whether these indices remain stable in the rank of the distribution in repeated measures (tracking) during perturbations of weight in obese children.

Design and Measurements: In a longitudinal study, 115 obese children were examined during a 12-week weight loss programme and 28 months of follow-up. Height, weight, body composition, Tanner stages, testicular size, time of menarche, and concentrations of leptin and SLR were measured at baseline, on days 14, 33, and 82, and from months 10, 16, and 28.

Results: During weight loss, leptin decreased and the SLR increased. During weight regain, leptin increased and the SLR decreased. The partial correlation coefficients expressing the relationship between leptin and SLR were significant in girls during both weight loss and weight regain, whereas in boys they were much weaker and not significant. Leptin, SLR and FLI exhibited individual-specific levels (tracking) during weight loss and regain in boys and girls. The observed tracking seemed stronger during weight loss than during weight regain. The observed tracking was independent of both baseline body mass index (BMI) standard deviation score (SDS) and pubertal development at baseline and of subsequent changes in BMI SDS and puberty stages.

Conclusion: Leptin and the SLR exhibit tracking during weight loss and regain, which indicates individual stability in the leptin system despite challenges of weight.

Introduction
In spite of the fairly high correlation between leptin and measures of the size of the body fat mass [1] during both weight-stable periods and during weight changes [2, 3], there remain considerable inter-individual differences for a given body fat mass [4]. These differences may be due to known inter-individual differences in other determinants of leptin concentrations, such as testicular size and testosterone [5], menarche [6], concentrations of cortisol, growth hormone [7], and insulin [8], and fasting, overeating, and exercise [9, 10]. Such determinants may vary over time, along with the size of the fat mass, or they may be stable over time irrespective of the size of the body fat mass or perturbations in it over time. Typically, such stable inter-individual differences may originate in individual differences in genetic profiles continuously influencing the phenotype over time or in earlier environmental exposures, perhaps even in the pre- or postnatal period, that lead to persistent influences, usually called programming. Twin studies have suggested that there may be a genetic influence on leptin levels [11–14], but these studies have not fully assessed to what extent this is explained by the genetics of the size of the body fat mass and if the genetic influence is stable over time. If there are such stable determinants, leptin would...
exhibit tracking over time, which can be quantified by the degree of correlation between serial measurements of these indices in the same individuals. Perfect tracking would give a correlation coefficient of 1.0 and no tracking a correlation coefficient of 0. Tracking of leptin means that concentrations of leptin in a group of individuals maintain the same (or a similar) ranking in the distribution in serial measurements. This means that the same individual would exhibit leptin concentrations in the same percentile band of leptin concentrations for the whole group, irrespective of changes in absolute leptin concentrations.

Intriguingly, it has been shown that over-expression of the soluble leptin receptor (SLR) in ob/ob mice enhances the effect of administered leptin, which suggests that the SLR might interact and contribute directly in leptin functioning [15]. If the SLR has an independent function in leptin action in humans, it should be anticipated to exhibit independent qualities. Knowledge of how much tracking of leptin, SLR, and the free leptin index (FLI) contributes to the development of these indices during weight loss and regain in the same individuals could prove valuable in the analysis of how these indices can be adapted in the clinical predictions of future weight changes.

The aim of the present study is to investigate if these indices show tracking over time during loss of weight induced by enforced modifications of the energy balance components and during the subsequent phase of weight regain. Further, the natural development of leptin during puberty is known to be especially affected by concomitant developments of testosterone and fat mass. Therefore, the analyses of leptin, SLR and FLI were separated by sex.

Material and Methods

Design

Groups of obese children were examined on days 1 (baseline), 14, 33, and 82 during the weight loss programme, and at months 10, 16, and 28 during the follow-up regimen. The examinations included interviews and assessment of Tanner stages (gonadal and pubertal), testicular size, weight, height, body composition by bio-impedance, and venous blood samples. The Scientific Ethical Committee of the County of Copenhagen approved the study (KA 97041), and written informed consent was obtained from all participants, as well as from their parents, according to the Helsinki Declaration and its later amendments.

Setting

The institution ‘Julemærkehjemmet’, Skælskør, Denmark offers a weight reduction programme consisting of a restricted low-fat diet with a fixed level of energy intake at 6,500–7,000 kJ per day. Follow-up examinations were carried out at the Paediatric Department at the University Hospital in Glostrup.

Children

During the study, 232 children were identified as eligible to participate, with 120 agreeing to do so. 90 children completed the weight loss programme, 68 engaged in follow-up, but only 44 children completed all examinations in the follow-up programme. For further details during weight loss and weight regain, please see [2, 3].

Procedures

Height was measured by a stadiometer to the nearest 5 mm. Weight was measured to the nearest 0.1 kg on a SECA Delta scale, model 707 (Simonsen and Weel). The body mass index (BMI) standard deviation score (SDS) was calculated by the least mean squares (LMS) method based on growth data generated in Danish boys and girls [16]. Fat-free mass (FFM) percentages were obtained by bioelectrical impedance spectroscopy using a multi-frequency impedance meter (SFB3; Uniqest Ltd., Australia) and calculated according to the equation proposed by Schaefer et al. [17], validated against total body potassium [17]. We also applied the equation of Wabitsch et al. [18], validated by deuterium dilution [18], which confirmed that changes in FFM were consistent with changes in total weight. The puberty stage was rated according to Tanner stages after evaluation of pubic hair and breasts, and testicular size was measured by Prader’s orchidometer.

Venous blood samples were collected from each child between 7:00 am and 8:30 am after an overnight fast during the weight loss regimen, and between 8:00 am and 9:00 am after an overnight fast during follow-up. The serum was frozen at –80 °C until analysis. The radioimmunoassay for leptin was performed 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%. The human leptin receptor enzyme-linked immunosorbent assay (ELISA) kit from Biovendor Laboratory Medicine (Brno, Czech Republic) was used and performed in duplicate, and showed an inter-assay coefficient of variation of 17.5% and an intra-assay coefficient of variation of 16.5%. Owing to relatively high variations, all samples were analysed in duplicate on two separate occasions. According to the manufacturer, the human leptin receptor ELISA measures total SLR, independent of the amount of leptin binding it. The FLI has been proposed as a marker of mainly free leptin in the circulation [19] and may be a better determinant of leptin function [20]. The FLI was calculated as leptin concentration divided by SLR concentration and multiplied by 100.

Statistical Methods

Leptin and SLR concentrations were log-transformed in order to achieve approximate normality.

Partial correlation coefficients (r) between log-transformed leptin and log-transformed SLR were adjusted for BMI SDS and puberty.

Tracking was investigated using partial correlation coefficients between 2 measures of leptin, SLR, and FLI in the same child adjusted for puberty and BMI SDS at both measurements.

Possible selection bias to drop-out was evaluated by testing whether subjects dropping out differed from those completing the study with respect to age and adiposity and whether re-analysis of subjects with no missing values gave other estimates [21, 22].

Possible selection bias to pubertal development was evaluated by repeating analyses where subjects progressing into puberty were censored.

Results

The children experienced a significant weight loss (–1.0 BMI SDS in boys (p < 0.0001) and –0.9 BMI SDS in girls (p < 0.0001)) and a significant weight regain (0.6 BMI SDS in boys (p < 0.0001) and 0.8 BMI SDS in girls (p < 0.0001)) during follow-up. The baseline characteristics of the children, as well as the developments of leptin and BMI SDS during weight loss and weight regain, have been reported previously [2, 3].

During the initial phase of weight loss (days 1–82) and during the initial phase of weight regain (day 82 to month 28), leptin and SLR showed an inverse pattern, where leptin de-
Tracking of BMI SDS was strong in both boys and girls during both weight loss and regain (table 1). Table 3 shows the leptin concentrations compared with other measures of leptin in the same child in boys and girls during weight loss and regain. Tracking of leptin appeared stronger in boys since the correlations were significant at a larger number of time points: 18 for boys compared to 7 for girls. In girls, tracking of leptin appeared stronger in weight loss than in regain since the correlations were significant at a larger number of time points: 6 (out of 6) for weight loss and 1.
Tracking of the FLI appeared strong in weight loss and in regain in both sexes: 11 significant correlation coefficients (out of 12) for weight loss in both sexes and 2 (out of 3) in both sexes for regain (table 5). The partial correlation coefficients imply adjustment for BMI SDS and puberty stages.

Figure 1a, b shows the partial correlation coefficients of leptin (solid line) and SLR (dotted line) with 95% confidence intervals (CI) during weight loss and regain in boys and girls. The correlation coefficients express the relationship compar-

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**p < 0.001; *p > 0.001 and p < 0.05; #p > 0.05.
Partial means adjustment for BMI SDS and puberty stages in the correlation analysis.

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** = p < 0.001; * = p > 0.001 and p < 0.05; † = p > 0.05.
Partial means adjustment for BMI SDS and puberty stages in the correlation analysis.

Tracking of the FLI appeared stronger in weight loss and in regain in both sexes: 11 significant correlation coefficients (out of 12) for weight loss in both sexes and 2 (out of 3) in both sexes for regain (table 5). The partial correlation coefficients imply adjustment for BMI SDS and puberty stages.

Figure 1a, b shows the partial correlation coefficients of leptin (solid line) and SLR (dotted line) with 95% confidence intervals (CI) during weight loss and regain in boys and girls. The correlation coefficients express the relationship compar-
In order to evaluate whether puberty development biased these findings, analyses were repeated while censoring out all children progressing into puberty. Pre-pubertal children were defined as girls before menarche and boys with a testicular volume below 7 ml. The analysis of only pre-pubertal children did not alter the relationships found regarding the partial correlation between leptin and the SLR and tracking of leptin and the SLR (data not shown).

**Discussion**

Novel findings of this longitudinal study are that leptin, SLR and FLI exhibit tracking during both weight loss and subsequent weight regain. Tracking of leptin and the SLR was independent of baseline BMI and puberty and subsequent changes of BMI and puberty.

A difference in tracking between boys and girls was suggested since leptin and FLI exhibited stronger tracking in boys than in girls. In girls, there was the tendency that tracking seemed stronger during weight loss than during regain, whereas this tendency was not apparent in boys. Further, the partial correlation coefficients expressing the relationship between leptin and SLR were significant in girls during both weight loss and weight regain whereas in boys they were not.

**Fig. 1.** Changes in the partial correlation coefficients of leptin (solid line) and SLR (dotted line) with 95% CI during weight loss and regain in (a) boys and (b) girls. The correlation coefficients express the relationship comparing baseline measures of leptin (solid line) with 6 subsequent measures of leptin in the same child and baseline measures of SLR (dotted line) with 6 subsequent measures of SLR in the same child. Concomitant measures of BMI SDS (thin dotted line) show weight loss and regain in boys. The partial correlation coefficients were adjusted for concomitant changes in BMI SDS and puberty stage.
were much weaker, also suggesting a difference between the genders.

The present study confirms a number of earlier findings. Weight loss is accompanied by decreasing leptin concentrations and increasing SLR concentrations in both genders [23–25], and the opposite effect is seen in weight regain in anorectics [20] and in malnourished children [26]. However, it should be appreciated that these studies are primarily cross-sectional or ‘before-and-after’ weight change studies, and do not offer the same possibility to evaluate concomitant changes during either weight loss or regain.

The longitudinal design of the present study had the implication that weight loss was a forced action in a controlled environment whereas the weight regain occurred in a free environment at home, where the energy balance seemed to restore a degree of obesity during follow-up that was comparable with pre-weight loss (baseline). Tracking seemed stronger in weight loss, though significant tracking was also observed during regain, which may suggest that the observed changes in the leptin axis seemed competent and not put out of order by either forced weight loss or during an intrinsic-restoration regulation towards weight regain.

In earlier studies in these children, it has been shown during weight loss in boys and girls that leptin declined more than anticipated from the cross-sectional associations between leptin and body weight. This suggests dissociation in the leptin-weight relationship that may contribute to the general variability in leptin concentrations observed in obese subjects [2]. During weight regain, proportional increases in leptin and BMI SDS were demonstrated, suggesting an intact leptin response during re-accumulation of fat. At the same time, it was shown that leptin concentrations were restored to a level aligned with pre-weight loss during continuous weight regain, which suggests an inefficient leptin control exerted by leptin on weight regain [3]. These findings suggest that leptin plays an active role in energy balance regulation, but that this effect may be attenuated during continuous weight loss. Therefore, it is intriguing that leptin and its soluble receptor show tracking within an individual, and especially so during challenges of weight in boys and girls, further suggesting a genetic influence compatible with an endocrine body weight regulatory system.

It is also intriguing that tracking seemed to be less pronounced with pre-weight loss (baseline). Tracking seemed stronger in weight loss, though significant tracking was also observed during regain, which may suggest that the observed changes in the leptin axis seemed competent and not put out of order by either forced weight loss or during an intrinsic-restoration regulation towards weight regain. Tracking seemed stronger during weight loss than during regain, whereas this was not obvious in boys. This effect may be due to sex differences in body composition development during puberty [34] or other considerations regarding methodology [35] that may affect leptin, SLR, or body fat depots. Therefore, the statistical analysis was repeated excluding those children entering puberty; however, censoring out pubertal development did not alter the relationships regarding tracking of either leptin or the SLR during either weight loss or weight regain previously found in the whole group.

A number of possible confounding influences on the conclusions should be acknowledged. In the present study, we sought to avoid the diurnal and discordant [36] changes in leptin and SLR concentrations [37], by sampling during fasting.

The SLR ELISA exhibited variability, which occurred even though the measurements were made in duplicate and were later repeated in duplicate. Nevertheless, the SLR concentrations showed significant tracking during changes of weight. Further, no indication was found that measurement uncertainty was unilaterally biased by gender, age or weight status. Therefore, any variability in the SLR ELISA results would have tended to result in an underestimation of the relationships established, since greater variability most likely would weaken the SLR correlations.

Attrition in numbers of participants during the study, though common in weight loss studies [38], always threatens to reduce the statistical power; however, significant findings were found. Nevertheless, attrition may have attributed to the finding that tracking was more pronounced in weight loss than...
in regain. Great effort was put into checking for possible selection bias by exclusion of participants with missing values in statistical re-analyses, which did not alter the correlation relationships in the present study.

According to the findings of tracking of leptin, SLR, and FLI during changes in weight in the present longitudinal study, serial measurements of leptin and SLR simultaneously provide information about the leptin control system and, in turn, energy status and flux. Such an approach might improve the clinical assessment using the leptin system of later weight developments [39]. However, this needs to be supported by information regarding normal ranges of leptin and the SLR during weight changes in childhood, as well as characterization of concentrations of leptin and the SLR with regard to maintenance of an individual stable weight in combination with other phenotypical traits (age, BMI SDS, puberty etc.) before these indices can be adapted in the clinical assessments of future weight changes.

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Disclosure Statement

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research project.

References


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