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Leptin Levels among Prepubertal Children with Down Syndrome Compared to their Siblings

Sheela N. Magge, M.D., M.S.C.E.^{1,3,4}, Kristen L. O'Neill, M.S.², Justine Shults, Ph.D.^{3,4}, Virginia A. Stallings, M.D.^{2,4}, and Nicolas Stettler, M.D., M.S.C.E.^{2,3,4}

¹ Division of Endocrinology and Diabetes, The Children's Hospital of Philadelphia

² Division of Gastroenterology, Hepatology, and Nutrition, The Children's Hospital of Philadelphia

³ Center for Clinical Epidemiology and Biostatistics

⁴ University of Pennsylvania School of Medicine

Abstract

Objectives—To compare levels of leptin and other obesity-related hormones between prepubertal children with Down syndrome (DS), a population at high obesity risk, and unaffected siblings, to better understand the pathophysiology of obesity in children with DS.

Study design—Cross-sectional study of 35 children with DS and 33 control siblings, ages 4 to 10 years, with a fasting blood sample, and anthropometric measurements to estimate body composition. Generalized estimating equations were used to account for the lack of independence between siblings.

Results—In addition to having higher body mass index (BMI) and percent body fat, children with DS had higher leptin levels than unaffected siblings, even after adjustment for age, sex, race, and ethnicity (difference: 5.8 ng/ml, 95% CI: 2.4–9.3, $p=0.001$), and further adjustment for percent body fat (difference: 2.7 ng/ml, 95% CI: 0.08–5.4, $p=0.04$). Leptin and percent body fat were positively associated in both groups ($p<0.0001$), but with a significantly greater positive association in the DS group, suggesting a significant effect modification ($p<0.0001$).

Conclusions—This group of children with DS had increased leptin for percent body fat than their unaffected siblings. This difference may contribute to the increased risk for obesity in children with DS.

Keywords

Obesity; Body Mass Index; Pediatrics; Body composition; Adiposity

Individuals with Down syndrome (DS) are at increased risk for several endocrinologic conditions, including hypothyroidism, growth retardation, diabetes mellitus, and obesity (3, 4). The reason for the increased risk of obesity in this population is unclear, but several mechanisms have been suggested, including a decreased resting metabolic rate (5,6) and

Corresponding author: Sheela N. Magge, M.D., M.S.C.E., Division of Endocrinology and Diabetes Rm 8416 Main Bldg, The Children's Hospital of Philadelphia, 34th and Civic Center Blvd, Philadelphia, PA 19104, Phone: 215-590-3618, Fax: 215-590-3053, Email: magge@email.chop.edu.

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differences in physical activity patterns (7). With the increase in the life expectancy of people with DS (3), obesity and related morbidity and mortality are emerging as important long-term consequences (3).

Adipokines such as leptin have been implicated in the pathophysiology of obesity. Leptin is a hormone secreted by adipocytes, acting in the hypothalamus to suppress appetite and regulate body weight (10). It is positively correlated with percentage of body fat (11); thus, it is postulated that obese individuals have some degree of leptin resistance (11). It is unclear whether this mechanism is also at work in children with DS.

The aim of the current study was to compare levels of leptin and other obesity-related hormones in prepubertal children with DS with a control group of unaffected siblings, to better understand the hormonal mechanisms of obesity in children with DS. In addition, we investigated the association between percentage body fat and leptin levels among children with DS and among their siblings. We hypothesized that leptin levels are higher among children with DS, and that the association between leptin levels and percentage body fat is different in children with DS compared to their siblings.

METHODS

The present cross-sectional study was performed as part of the baseline visit of a prospective study. Families were recruited through referring physicians and DS parent support groups in the greater Philadelphia area. Thirty-six families were enrolled between November, 2001 and June, 2003. Inclusion criteria for participants were: (1) at least two children per family, one with DS and one unaffected child, (2) ages 4–10 years, (3) prepubertal, and (4) BMI 5th to 95th percentile for age and sex (13), based on the parent's telephone report of their children's weight and height. The final sample did, however, include some children with a BMI > 95th percentile, based upon the measured height and weight on the day of the baseline visit. Exclusion criteria were history of: (1) cancer, including leukemia, (2) congenital heart disease necessitating open heart surgery, (3) intestinal resection, (4) hypothyroidism requiring thyroid hormone replacement therapy, or (5) other chronic conditions affecting growth or energy balance. In two families, more than one eligible child without DS was eligible. In those cases, the caregiver decided which unaffected child would participate based on the child's interest. Written consent and assent were obtained from all subjects before participation, and the study was approved by the Institutional Review Board of The Children's Hospital of Philadelphia.

Questionnaires completed by the parents were used to collect the participants' dates of birth, and self-reported race and ethnicity. Anthropometric measurements were performed at The Children's Hospital of Philadelphia General Clinical Research Center by trained research anthropometrists using standard methods (14). Weight was measured with the subject wearing a light gown without shoes by use of a Scaletronix digital scale (Scaletronix, White Plains, NY), calibrated daily. Height was measured using a wall-mounted stadiometer (Holtain Inc., Crymych, UK). Body mass index (BMI) was calculated as weight in kilograms divided by height in meter squared. BMI z-scores were calculated using age and sex specific BMI reference data, as recommended by the Centers for Disease Control and Prevention (15). Triceps, biceps, subscapular, and supra iliac skinfolds were measured using standard techniques (14). Measurements were repeated three times, and average values utilized. These measurements were used in sex-specific equations validated in children without DS, to calculate fat mass, fat-free mass, and percent body fat (16,17). Fat mass, fat-free mass, and percent body fat were also measured by dual energy X-ray absorptiometry (DXA) in a sub-sample of subjects who were sufficiently cooperative to obtain quality data (24 subjects with DS, 32 control siblings). The primary analysis was performed using skinfold thickness-derived body composition, and a confirmatory secondary analysis was performed in the sub-sample

with DXA data. Because DS is associated with decreased height, and because fat-free mass and fat mass are in part related to height, we also calculated the fat-free mass index (fat-free mass/height²), and fat mass index (fat mass/height²) to control for height.

After a supervised twelve-hour overnight fast, a blood sample was drawn for leptin, ghrelin, insulin, glucose, TSH, T3, T4, reverse T3, IGF-1, and IGF-BP3. Fasting insulin and glucose levels were used to calculate the QUICKI (quantitative insulin sensitivity check index = $1 / \{ \log \text{fasting insulin (uIU/mL)} + \log \text{fasting glycemia (mg/dL)} \}$) and HOMA (homeostasis model assessment = $[\text{fasting insulin (uIU/mL)} * \text{fasting glycemia (mmol/L)}] / 22.5$), which are validated measures of insulin resistance in children (18,19).

Generalized estimating equations (GEE), as implemented in Stata 9.0, were used in the analysis to account for the lack of independence between subjects who were siblings. First, descriptive analyses of the two groups were performed. This included means and standard deviations for continuous variables and frequencies for categorical variables. In addition, unadjusted and adjusted comparisons of demographic variables, anthropometric measures, and hormone profiles were performed between the two groups. Racial distribution and ethnicity were also compared between subjects with DS and controls, using the Fisher's exact test. The adjusted comparisons were made by modifying the GEE models to also include age, sex, race, and ethnicity. To explore variables in the pathway between DS and leptin levels, the main effect of DS status on leptin was also investigated with further adjustment for percent body fat, or, in an alternative confirmatory model, both fat mass and fat free mass. Finally, the relationship between leptin and percent body fat was examined. For this association, possible effect modification by DS status was assessed. This was done by considering a GEE model with leptin as the outcome and the following explanatory variables: (i) an indicator variable, I(DS), for DS status that took value one for subjects with DS, and zero otherwise, (ii) percent body fat, and (iii) a DS status \times percent body fat interaction term. If the regression coefficient associated with the interaction term differed significantly from zero, this would indicate that the linear relationship between leptin and percent body fat differed significantly between the DS subjects and their siblings. As for the earlier comparisons, the GEE interaction models were then further modified to include adjustment for race, sex, and ethnicity. The slope of the regression line (with 95% confidence interval) of leptin on percent fat was obtained for each group using the lincom procedure in Stata 9.0. The interaction analyses were then replicated with adjustment for both fat free mass and fat mass, instead of percent body fat. Also as a confirmatory analysis, all statistical tests were repeated using DXA-derived body composition data in the sub-sample for which these data were available. As graphical examination suggested that the association of leptin and percent body fat might not be linear, but rather curvilinear, in post hoc analysis the GEE interaction model was modified to include percent body fat, percent body fat squared, and the DS status \times percent body fat squared interaction term. We again also adjusted for age, race, sex, and ethnicity. A goodness of fit test specific for GEE (20) was applied to compare the fit of the models used to describe the relationship between leptin and percent body fat.

All statistical analyses were performed using STATA software (version 9.0; Stata Corp., College Station, Texas). Two-sided tests of hypotheses were considered, and a p-value < 0.05 was considered statistically significant.

RESULTS

Among the 72 eligible study participants, one subject with DS was excluded because of the discovery during the baseline visit of significant hypothyroidism (TSH=151 uIU/ml) requiring hormone replacement therapy (one of the exclusion criteria), and three control siblings were excluded because they refused the blood draw. Thus, data from 35 subjects with DS and 33

control siblings were available for analysis. Leptin levels, BMI z-score, and percent body fat were significantly higher among the children with DS than among unaffected siblings (Table). This was true despite the fact that, by design, most subjects were not obese. Fat-free mass was higher among the unaffected siblings, likely due to the fact that DS is associated with decreased height. Both fat-free mass index and fat mass index were significantly higher in the children with DS. To confirm our analyses (shown in Table) using percent body fat, fat mass, and fat-free mass calculated from skinfold measurements (equations validated in children without DS (16,17)), we repeated these analyses using percent body fat, fat mass, and fat-free mass data obtained from DXA in the sub-sample of children who were able to tolerate the procedure. Again, percent body fat was significantly higher in children with DS than unaffected siblings, fat-free mass was higher in unaffected siblings, and fat mass was not significantly different between the groups.

Interestingly, there was no significant difference between the groups in ghrelin, an appetite-enhancing hormone (21). There was also no significant difference in insulin, glucose, measures of insulin resistance (HOMA and QUICKI), or of IGF-1 and IGF-BP3 (suggestive growth hormone axis function). Although children requiring treatment for hypothyroidism were excluded by design, TSH values were higher among children with DS than among the controls, within the normal range. Surprisingly, T3 levels were also higher in the DS group, although again within normal limits. After comparison of the two groups with adjustment for age, sex, race, and ethnicity, the results remained essentially the same, except for glucose level, which became significantly higher among the children with DS compared to unaffected siblings ($p=0.007$) (Table).

To explore whether the differences in leptin levels between the two groups (unadjusted difference 4.9 ng/ml, 95% CI: 1.2–8.6, $p=0.009$; difference adjusted for sex, age, race, and ethnicity 5.8 ng/ml, 95% CI: 2.4–9.3, $p=0.001$), were explained by differences in body composition, the analyses were repeated with additional adjustment for percentage body fat. Leptin levels remained significantly higher among the children in the DS group (adjusted difference 2.7 ng/ml, 95% CI: 0.08 – 5.4, $p=0.04$). In an alternative confirmatory model, the analyses were performed with adjustment for both fat-free mass and fat mass, instead of percent fat. In this model, leptin levels also remained significantly higher among the subjects with DS (adjusted difference 4.6 ng/ml, 95% CI: 1.3 – 7.9, $p=0.006$).

The secondary aim of this study was to investigate whether the association between leptin and percentage body fat was different in children with and without DS. As expected, these two variables were positively associated in the unadjusted analysis for the entire sample of children with and without DS (coefficient for percent body fat 1.1, 95% CI: 0.9 – 1.2, $p<0.0001$). In the regression analysis of the relationship between leptin and percentage body fat, an interaction factor by DS was significant ($p<0.0001$), indicating effect modification by DS status on this association. Among the children with DS, percentage body fat was positively associated with leptin levels in the unadjusted analysis (coefficient 1.3, 95% CI: 1.1 – 1.6, $p<0.0001$), and in the analysis adjusted for age, sex, race, and ethnicity (coefficient 1.3, 95% CI: 1 – 1.5, $p<0.0001$). Among the unaffected controls, percentage body fat was also positively associated with leptin levels in the unadjusted analysis (coefficient 0.7, 95% CI: 0.5 – 0.9, $p<0.0001$), and in the analysis adjusted for age, sex, race, and ethnicity (coefficient 0.6, 95% CI: 0.4 – 0.8, $p<0.0001$). As indicated by the significance of the interaction term and the non-overlapping 95% CI, the positive relationship between leptin and percent body fat in the two groups were statistically different. In secondary analyses, fat mass and fat-free mass together, instead of percent body fat, were used and the results were essentially the same. Because of the curvilinear appearance of the data, the GEE interaction model was modified to include percent body fat, percent body fat squared, and the DS status \times percent body fat squared interaction term, in order to see whether a second order model better described this relationship. For the first and

second order models, both unadjusted and adjusted for age, sex, race, and ethnicity, the results remained similar, again with significant interaction terms ($p < 0.001$). When a goodness of fit test specific for GEE (20) was applied to the models adjusted for demographic variables, there was a slightly greater R^2 in the second order model, $R^2 = 0.82$, as compared to the linear model, $R^2 = 0.79$. Therefore, the second order model was used as the final model to describe the relationship between leptin and percent body fat (Figure).

DISCUSSION

As hypothesized, we found that leptin levels were higher in prepubertal children with DS, a population at high risk for obesity, compared to a control group of unaffected siblings. This was true despite the fact that severely obese children were excluded from the study by design. The higher leptin levels in children with DS could not be fully explained by percent body fat. Leptin and percent body fat were positively associated in both groups, but with a significantly greater positive association among the children with DS, best approximated by a second order model (Figure). This finding indicates increased leptin secretion for a given percent body fat among children with DS compared to unaffected siblings.

This study reports the elevated leptin levels in children with DS, a group known to be at increased risk for obesity. In general, obese individuals have higher leptin levels than normal weight individuals, and it is believed that exogenous obesity is associated with leptin resistance (11). This study also reports a difference in the magnitude of the association between leptin and adiposity in individuals with DS compared to controls. This is a distinct finding from a difference in baseline levels or set points, and may represent increased leptin resistance in children with DS, if substantiated by future studies. The steeper slope in the DS group may suggest a syndromic model of more severe leptin resistance. In another genetic syndrome with a predisposition to obesity, Prader Willi Syndrome (PWS), the association between leptin and total body fat had an increased slope in subjects with PWS compared to controls, similar to our findings in DS (22). In two other studies, however, no difference in leptin levels were observed between subjects with PWS and controls (23,24), even after adjusting for adiposity (22). Thus, there are conflicting data about PWS as a possible syndromic model of increased leptin resistance.

There is precedence for variability in the amount of leptin produced by a given amount of adipose tissue. Women have been found to have 2- to 3-fold higher leptin levels for the same amount of fat compared to men (25), and graphical observation of some data, not confirmed by statistical analyses, suggest that the association between fat mass and leptin may differ between men and women (25,26). As women have proportionally more subcutaneous and less visceral fat than men, fat distribution could be postulated to cause the sexual dimorphism in serum leptin levels. However, fat distribution does not completely explain the leptin sex difference (26,27). Some have postulated that increased circulating leptin levels in women are at least in part due to the effect of sex hormones on leptin production, and may also involve genetic differences between the X and Y chromosomes (26,27). Given that the children in our study were prepubertal, it is not surprising that we did not find sex to be an effect modifier of the relationship between leptin and percentage body fat (data not shown). However, there have been conflicting data on whether even prepubertal girls have elevated levels of leptin compared to boys, after adjusting for adiposity (26–28).

Just as there is an association between increased visceral fat (as opposed to subcutaneous fat) or central/truncal fat (as opposed to peripheral/extremities fat), and increased insulin resistance (29,30), as suggested above, the anatomic distribution of fat may also have implications on leptin secretion. Van Harmelen et al performed in vitro studies on fat tissue from biopsies in women. They found that leptin secretion rates (leptin secretion per lipid weight or cell number)

were two to three times higher in subcutaneous fat tissue as compared to omental (visceral) fat tissue (31). Also, subcutaneous fat cells were approximately 50% larger than omental fat cells. Fat cell size and leptin secretion rates were positively correlated in both fat stores. However, there have been mixed results in studies of the impact of the distribution of adipose tissue on leptin secretion. The present study was not designed to study this question, so visceral fat was not measured and no fat biopsy or functional leptin secretion testing was performed.

Similar to our findings, different slopes for the correlation between leptin and body fat have also been demonstrated in molecular studies of rats. Zhang et al (32) found that rats with heterozygous leptin receptor deficiency had a plasma leptin concentration per gram fat mass that was intermediate between wildtype rats and rats with homozygous leptin receptor deficiency. These authors demonstrate different slopes of the correlation lines, supporting the hypothesis that leptin regulates its own secretion via effects on the adipocyte. The authors suggest that dysregulation of leptin synthesis in adipocytes may cause differences in humans in the amount of leptin secreted per unit fat. However, the brain is also felt to be involved in leptin regulation. Transgenic complementation of leptin receptor in mice with homozygous leptin receptor deficiency partially rescued the effects of leptin deficiency in these mice, and resulted in increased expression of regulating hypothalamic genes (33). Thus both central nervous system and direct peripheral actions of leptin are thought to be involved in the regulation of leptin secretion. Our human model of increased leptin secretion by percentage body fat among children with DS may contribute to improving the understanding of leptin regulation in humans.

The reason for increased leptin for percentage body fat in children with DS is unclear. It is unlikely to be related to the growth hormone axis or pubertal hormones, given that these children were all prepubertal (and that there are no known differences in growth hormone levels in children with DS compared to unaffected children). The present study was one of the few to report hormone levels of healthy children with DS compared to their siblings, and no differences in ghrelin or insulin were observed between the two groups of children. These data suggest that these obesity-related hormones do not explain the differences in leptin levels between children with and without DS. However, the relative small size of our study may have limited our ability to detect existing differences, and larger studies are needed to better understand the obesity-related hormone milieu of individuals with DS. Considering that the present study was conducted among siblings and that children with DS have three copies of chromosome 21, a genetic basis to more severe leptin resistance with DS should be considered. As leptin research continues, it is important to identify populations with a genetic or hormonal predisposition to increased leptin secretion for percentage body fat, possibly suggesting increased leptin resistance. Children with DS may be an example of such a population.

The present study had some limitations. Our sample size was limited, and our findings of increased leptin secretion for percentage body fat should be replicated in larger samples. Our limited sample size could have also explained the fact that insulin and glucose were both higher in the DS group (although only significant in the adjusted analysis for glucose), but our measure of insulin resistance, HOMA and QUICKI, were not statistically different between the two groups. With larger numbers, the differences in these measures between the two groups might have reached statistical significance. Further, having a few children with extreme obesity could have influenced our results, although we feel this is unlikely given that there were such individuals in both groups. As in all cross-sectional studies, this study could not address the issue of causation. Furthermore, in the main analysis, adiposity was measured by skinfold thickness, a method validated in children without DS, but not in children with DS. Finally, the study was not designed to measure the contribution of the pattern of adipose tissue distribution. This may be a useful direction for future research in this population.

The strengths of the study included the presence of a control group, particularly one consisting of siblings, thus decreasing the potential for confounding by environmental and genetic factors, and decreasing the recruitment bias of the healthy controls. By choosing subjects who were, by design, mostly not obese, we focused the study on risk factors for obesity predating the obesity onset. In addition, choosing prepubertal subjects allowed us to study relationships not confounded by pubertal stage. Finally, anthropometric variables were measured by highly-trained research staff, contributing to the quality of this data, and our findings were robust using several confirmatory analyses.

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LIST OF ABBREVIATIONS

DS	Down syndrome
BMI	body mass index
QUICKI	quantitative insulin sensitivity check index
HOMA	homeostasis model assessment
PWS	Prader Willi Syndrome
DXA	dual energy X-ray absorptiometry

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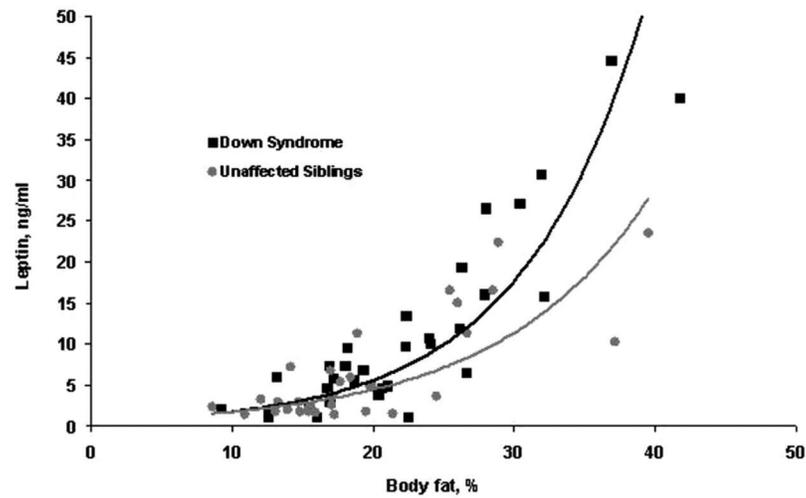


Figure. Relationship between leptin and percent body fat, by Down syndrome status, in a sample of 35 prepubertal children with Down syndrome and 33 prepubertal healthy siblings. Data from children with Down[H1] syndrome are shown with dark squares, and siblings are shown with light circles. Second order trend lines for each group are shown.