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ORIGINAL ARTICLE

Reference values for serum leptin in healthy non-obese children and adolescents

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ABSTRACT

Background: Adipokines are biologically active, low-molecular weight peptides, which play a major role in metabolic homeostasis in humans. Leptin has gained increasing attention in pediatrics as a biomarker for various metabolic pathologies. Yet, its usefulness is hampered by the relative lack of reference values from pediatric settings. Accordingly, this study aims to evaluate serum concentrations of leptin, soluble leptin receptor (sOB-R), and free leptin index (FLI) in healthy Danish schoolchildren aged 6–18 years and subsequently to establish reference intervals across sex and age groups.

Methods: A total of 1193 healthy, non-obese Danish schoolchildren (730 girls, 463 boys) aged 6–18 years (median 11.9) were examined by trained medical staff. Serum leptin and sOB-R concentrations in venous fasting blood samples were quantitated by immunoassay. Percentile curves of leptin, sOB-R, and free leptin index were calculated using the General Additive Model for Location Scale and Shape (GAMLSS).

Results: Significant age and sex-dependent differences in circulating leptin levels were found. In boys, the median leptin concentration for all ages combined was 3.35 μg/L (95%-interval: 0.71–22.47) and in girls, it was 9.89 ng/L (95%-interval: 2.06–41.49). For SOB-R, no sex-specific difference was found, and the median sOB-R concentration was 8.24 μg/L (IQR: 3.58–23.74; range: < 1.56–744.15).

Conclusion: We demonstrated an age-dependent correlation with both serum leptin concentration and free leptin index with a gradual and significant increase in girls throughout childhood and adolescence and a significantly higher leptin concentration and free leptin index bell-shaped peak in early adolescence in boys.

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Introduction

Adipose tissue is an important endocrine organ which secretes several biologically active, low-molecular weight peptides, or adipokines, which play a major role in human metabolic homeostasis [1]. Leptin is an important adipokine that is synthesized mainly, but not exclusively, by white adipose tissue and has vast endocrine and metabolic functions [1,2]. Leptin is involved in the regulation of feeding and energy balance [3,4] and serves as a mediator of the neuroendocrinological adaptation to fasting by influencing the expression of different neuropeptides that regulates energy expenditure [2,5]. Furthermore, leptin is implicated in fatty acid metabolism [6], in the reproductive system [7,8], in the immune system [9–11], and the serum leptin concentration has been proposed as a predictor of metabolic risks such as insulin resistance [12]. Accordingly, leptin is increased in obese males and females compared to non-obese individuals [13,14].

Importantly, the bioavailability of leptin is modulated by the soluble leptin receptor (sOB-R) [15]. Leptin achieves signal transduction via four different receptor (OB-R) isoforms, but the amount of functionally active OB-R is affected by constitutive shedding of the extra-cellular domain [16]. The product of the cleavage process, i.e. the sOB-R, is the main binding protein for leptin [17] and thus modulates its bioavailability [18]. It has been proposed that the ratio between leptin and sOB-R, or the free leptin index (FLI), may be a useful tool to assess leptin activity [19].

Leptin has gained increasing attention in pediatrics as a serum biomarker due to its correlation to various metabolic risk factors such as insulin resistance, metabolic syndrome, and cardiovascular disease [20–22]. Childhood obesity has increased dramatically across ethnic groups in recent decades, and with that the obesity-associated comorbidities have risen in importance [23]. Consequently, the need for cardiometabolic risk stratification has increased and created a demand for pediatric reference values for relevant biomarkers, including leptin, the sOB-R, and their mutual relationship.

However, the interpretation of serum values in children, and thus an assessment of the clinical utility of the analyte, is hampered by the relative lack of reference values from a pediatric setting. Contrary to adults, only a few studies on

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children and adolescents have been published [24]. Furthermore, the available data is largely obtained in small and heterogeneous study materials containing a limited number of healthy children [25].

The objective of the present study is to elucidate the naturally occurring serum concentrations of leptin and sOB-R and calculate the FLI in a large population-based sampled cohort of healthy, non-obese Danish schoolchildren and furthermore, to establish reference intervals for children and adolescents across sex and age groups.

Material

Subjects

Danish schoolchildren from several municipalities in the region of Zealand, Denmark, were included between October 2010 and November 2013. Exclusion criteria were obesity defined as a body mass index (BMI) above the 95th percentile for sex and age [26] at the time of inclusion, and known diseases requiring regular medication. All participants and/or parents signed informed consent. The study was approved by the Danish Data Protection Agency (REG-06-2014) and the Regional Scientific Ethics Committee (protocol no. SJ-104).

Anthropometric measurements

The participants were examined by skilled research assistants immediately prior to blood sampling. Children's heights were measured using a stadiometer to the nearest millimeter with the subject standing without shoes. The weight was measured using a BC-418 Segmental Body Composition Analyzer (Tanita, Tokyo, Japan) to the nearest 100 grams; with subjects wearing light indoor clothes and no shoes. The degree of baseline BMI is expressed in standard deviation score (SDS) in order to adjust for age- and sex-related variation in reference intervals. BMI SDS was calculated by the LMS method by converting BMI into a normal distribution by sex and age using the median coefficient of variation and a measure of the skewness [27] based on the Box-Cox power plot from Danish BMI charts [28].

Additional data collection

Additional data on health and socioeconomic status were obtained through a structured family-based questionnaire. Similarly, the pubertal developmental stage was assessed by a questionnaire where standardized pictures of the five pubertal developmental stages suggested by Marshall and Tanner [29,30] are depictured accompanied by a text describing each category and thereby allowing the participants and their parents in estimating the appropriate pubertal stage *ad modum* Tanner

Biochemical analyses

After an overnight fast, venous blood samples were collected from each child between 07:00 and 09:00 h. The samples were processed immediately and the serum was stored at −80 °C until further analysis. Serum leptin concentration was quantitated *in singlo* using an optimized version of the Human Leptin ELISA Development Kit, DuoSet (DY398, R&D Systems, Minneapolis, MN, USA) following appropriate sample dilution. The detection limit of the assay was 0.0312 µg/L. Assay calibrator was highly purified *Escherichia coli* expressed recombinant human leptin produced by the manufacturer (AF398, R&D Systems, Minneapolis, MN, USA).

The sOB-R was quantitated *in singlo* using the Quantikine Human Leptin sR Immunoassay (DOBR00, R&D Systems, Minneapolis, MN, USA) as described by the manufacturer following appropriate sample dilution. The smallest sOB-R concentration detectable within a linear standard curve was 1.56 µg/L when the serum was diluted 1:4. When standards were diluted in serum to extend the standard curve, the dilution curve was not linear, suggesting a matrix-effect on the measurements. Assay calibrator was highly purified NS0-expressed recombinant leptin R/Fc chimera produced by the manufacturer.

For both leptin and sOB-R, the intra- and inter-assay coefficients of variations were <5% and <10%, respectively. At room temperature, leptin concentrations in serum were stable for 48 h, and sOB-R was stable for 56 h. Both analytes were stable for three months at −20 °C, and for 10 freeze-thaw cycles.

Statistical analyses

Statistical modelling was performed using the statistical software R, version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria). Skewness was estimated by D'Agostino test and log transformation of data was performed when appropriate. Differences between sexes were evaluated non-parametrically for the entire group and for each age interval using the Wilcoxon-Mann-Whitney test. Similarly, Wilcoxon-Mann-Whitney tests were used to compare specific age groups to the defined reference group of 16–18-year-old adolescents. As studies have demonstrated that healthy girls experience a graduate increase in body fat percentage throughout childhood and adolescence whereas healthy boys have a more stable body composition and often even decreasing fat percentage [31], the data was analyzed separately for each sex and subsequently adjusted for age and BMI SDS. As concentrations of sOB-R can provide an indication of free leptin, the free leptin index (FLI) being defined as the ratio of leptin to sOB-R [32] was calculated as [leptin]/[sOB-R] × 100.

Percentile curves of leptin, sOB-R, and FLI were calculated as a function of age as a continuous variable using the General Additive Model for Location Scale and Shape (GAMLSS) [33]. Percentiles for age groups applied exactly for the midpoint of each age group were equally calculated with GAMLSS. Furthermore, the correlation between leptin, sOB-R, and FLI and age was evaluated using a generalized linear model (GLM), adjusted for BMI SDS.

To avoid left censoring of data in cases of values below the limit of detection (LOD), we used a tobit regression (TOBIT) model [34] instead of the more traditional approach of substitution method of replacing the values below LOD by

Table 1. Percentiles of leptin (μg/L) calculated with the General Additive Model for Location Scale and Shape (GAMLSS).

	Percentiles for girls							Percentiles for boys						
Age (years)	2.5	5	25	50	75	95	97.5	2.5	5	25	50	75	95	97.5
6	0.92	1.19	2.53	4.18	6.88	14.45	18.61	0.44	0.55	1.15	1.97	3.47	8.22	11.03
7	1.09	1.40	2.93	4.80	7.83	16.09	20.52	0.54	0.68	1.37	2.29	3.94	9.03	11.99
8	1.29	1.65	3.40	5.52	8.93	17.99	22.75	0.68	0.84	1.65	2.72	4.59	10.25	13.53
9	1.54	1.95	3.96	6.37	10.21	20.23	25.37	0.83	1.02	1.99	3.26	5.49	12.30	16.23
10	1.82	2.30	4.60	7.34	11.65	22.71	28.29	0.99	1.23	2.41	3.99	6.82	15.70	20.96
11	2.14	2.69	5.32	8.42	13.26	25.45	31.51	1.14	1.41	2.84	4.81	8.45	20.54	28.03
12	2.54	3.18	6.21	9.76	15.24	28.85	35.51	1.10	1.38	2.88	5.04	9.21	24.11	33.91
13	3.04	3.79	7.32	11.43	17.71	33.06	40.48	0.88	1.11	2.40	4.35	8.32	23.80	34.73
14	3.58	4.44	8.49	13.16	20.24	37.30	45.45	0.66	0.84	1.86	3.44	6.75	20.53	30.78
15	4.07	5.04	9.53	14.66	22.38	40.74	49.42	0.55	0.69	1.51	2.77	5.41	16.45	24.73
16	4.46	5.50	10.30	15.73	23.84	42.90	51.81	0.51	0.64	1.34	2.37	4.45	12.65	18.57
17	4.71	5.79	10.75	16.31	24.53	43.65	52.50	0.52	0.64	1.24	2.07	3.63	9.16	12.81
18	4.84	5.94	10.92	16.46	24.59	43.27	51.84	0.53	0.63	1.13	1.77	2.88	6.33	8.41

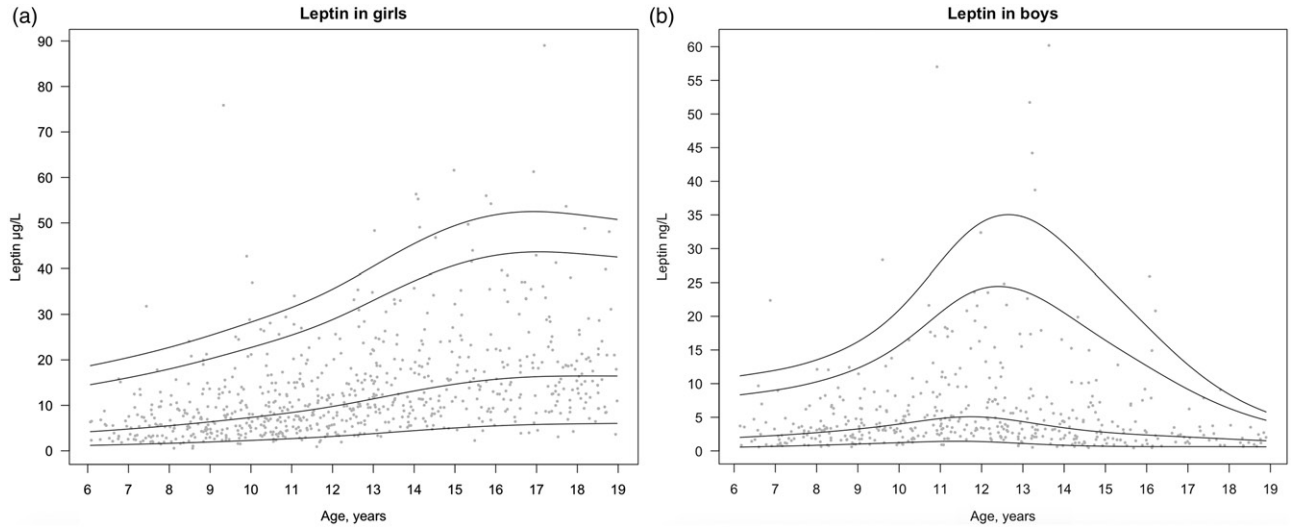

Figure 1. (a) Leptin (y-axis) in healthy girls as function of age (x-axis). The 97.5th, 95th, 50th, and 5th percentiles calculated with GAMLSS are presented. (b) Leptin (y-axis) in healthy boys as function of age (x-axis). The 97.5th, 95th, 50th, and 5th percentiles calculated with GAMLSS are presented.

a fixed valued [35] as the TOBIT approach has been reported to perform better when the proportion of non-detects, i.e. values below LOD, is below 30% [36].

Results

Included patients

A total of 1196 healthy children (730 girls and 463 boys) aged 6–18 (median 11.9) years old were included in the study. The children had normal weight (median 42.5 kg, range 18.4–85.7) for height (median 154.0 cm, range: 111.6–195.0) conveying a median BMI SDS of 0.34 (range: −1.65 to +1.65). There were no statistically significant associations with social class or BMI SDS between sex and age groups (data not shown).

Leptin

The combined leptin concentration for all included children was non-normally distributed (Shapiro-Wilk test, $W = 0.7729$, $p < 2.2e\text{-}16$) with a combined median for both genders and all ages of 7.06 μg/L (IQR: 3.12–13.49; 95%-interval: 0.87–29.56; range: 0.45–89.01). The leptin concentration was found to be significantly elevated in girls when compared to boys (Mann-Witney Wilcox U test, $p < 0.0001$) throughout the age groups, and data was subsequently analyzed separately for each sex.

In girls, the median leptin concentration for all ages combined was 9.89 μg/L (IQR: 5.69–16.92; 95%-interval: 2.06–41.49; range 0.55–89.01) and in boys, it was 3.35 μg/L (IQR: 1.90–6.42; 95%-interval: 0.71–22.47; range 0.45–60.19).

The calculation of reference intervals was performed in a GAMLSS model using age as a continuous variable and is presented in Table 1 and Figure 1(a,b).

In a generalized linear model, the total serum leptin concentration was correlated to age in girls ($p < 0.0001$), as leptin concentrations increased with age. For boys, serum leptin concentration exhibited a bell-shaped correlation to age with a maximum at 12.5 years as demonstrated by a generalized 2nd order polynomial regression model ($p < 0.0001$). When adjusted for BMI SDS, i.e. the degree of obesity, this age-dependent association persisted in both girls ($p < 0.0001$) and boys ($p < 0.0001$).

sOB-R

The combined median sOB-R concentration for all included children was 8.24 μg/L (IQR: 3.58–23.74; range: <1.56–744.15).

Table 2. Percentiles of sOB-R (μg/L) calculated with General Additive Model for Location Scale and Shape (GAMLSS). Values <1.56 μg/L are derived from TOBIT regression. See text for details.

	Percentiles for girls							Percentiles for boys						
Age (years)	2.5	5	25	50	75	95	97.5	2.5	5	25	50	75	95	97.5
6	1.02	1.62	5.46	12.61	36.22	408.65	1229.99	1.74	2.11	4.46	9.46	27.87	332.50	995.86
7	1.70	2.25	5.56	12.03	34.57	402.03	1217.60	1.65	2.00	4.28	9.14	27.23	335.43	1021.19
8	1.72	2.19	5.08	10.83	31.12	364.33	1105.25	1.24	1.60	3.85	8.49	25.47	317.08	975.52
9	1.52	1.94	4.49	9.55	27.39	319.94	970.56	1.14	1.49	3.67	8.14	24.49	308.26	955.57
10	1.33	1.71	3.97	8.44	24.14	281.16	852.83	1.43	1.75	3.80	8.21	24.81	321.44	1007.02
11	1.31	1.63	3.63	7.64	21.85	255.65	776.46	1.26	1.55	3.49	7.80	24.79	369.31	1242.48
12	1.30	1.59	3.38	7.04	20.13	236.81	720.27	1.26	1.55	3.49	7.80	24.79	369.31	1242.48
13	1.25	1.51	3.15	6.53	18.66	219.77	668.90	1.17	1.45	3.36	7.73	25.78	438.62	1578.37
14	1.18	1.43	2.96	6.11	17.44	205.07	624.30	1.09	1.36	3.22	7.61	26.41	502.14	1916.26
15	1.12	1.35	2.80	5.78	16.44	192.97	587.5	1.05	1.31	3.11	7.33	25.30	479.01	1830.05
16	1.07	1.29	2.67	5.50	15.63	183.04	557.32	1.07	1.32	3.02	6.90	22.73	383.19	1384.66
17	1.03	1.24	2.56	5.28	14.95	174.65	531.79	1.10	1.35	2.94	6.42	19.80	282.02	938.83
18	1.00	1.20	2.47	5.08	14.36	167.28	509.35	1.14	1.38	2.87	5.97	17.20	206.07	631.01

A total of 115 children (9.6%) had values below the LOD of 1.56 μg/L. An attempt to further expand the lower detection limit though dilutions of the calibrator proved futile due to the matrix effect [37,38]. An estimation of the values was calculated using TOBIT regression. The data was non-normally distributed (Shapiro-Wilk test, $W = 0.34$, $p < 2.2e\text{-}16$). No difference in sOB-R concentrations between sexes was demonstrated when using a non-parametric Wilcoxon-Mann-Whitney test ($p = 0.47$), a finding which is in accordance with a previous report by Kratzsch et al. [19].

The calculation of reference intervals was performed in a GAMLSS model using age as a continuous variable and is presented in Table 2.

In a generalized linear model, no statistic indication of age-dependent correlation was found in sOB-R, regardless of adjustment for BMI SDS, in girls ($p = 0.21$) or boys ($p = 0.81$). A non-parametric test (Spearman's rank correlation coefficient) demonstrated no correlation between age and sOB-R in girls ($p = 0.57$) or boys ($p = 0.37$).

The sub-population of children with sOB-R values below LOD did not differ from the rest of the population in regards to sex, social class, or BMI SDS (Wilcoxon-Mann-Whitney test; $p = 0.87$, $p = 0.60$, and $p = 0.98$, respectively), but had a higher median age ($p = 0.0001$) and leptin concentration ($p = 0.0152$).

Free-leptin index, FLI

The empirically observed median values for leptin concentration and FLI were calculated independently in each age-group and their course was found to be correlated for both girls and boys (Spearman's rank correlation coefficient, rho 0.49, $p < 0.0001$ and rho 0.48, $p < 0.0001$, respectively) and is presented in Figure 2(a,b). The calculation of smoothed reference intervals was performed in a GAMLSS model using age as a continuous variable and is presented in Table 3.

In girls, both leptin concentration and FLI increased significantly during childhood and adolescence and an adjusted GLM model demonstrated a relationship between age and FLI ($p < 0.0001$). Similarly, comparison of individual age groups to late adolescence, i.e. ages 16–18, demonstrated significantly lower FLI values in the younger age groups (i.e. ages 6–10, $p < 0.0001$; ages 11 and12, $p < 0.01$; and ages 13, $p < 0.05$; Wilcoxon-Mann-Whitney) when compared to late adolescence, i.e. ages 16–18. In boys however, a GLM model for all ages, showed no significant correlation to age when observing all age groups ($p = 0.947$), but were present when observing boys <14 years ($p < 0.01$). Accordingly, both leptin concentrations and FLI in boys were significantly higher during early adolescence (i.e. ages 10, 11, and 12; $p = 0.049$, $p = 0.014$, and $p = 0.005$, respectively) when compared by a Wilcoxon-Mann-Whitney test to late adolescence, i.e. ages 16–18.

Relation with pubertal stage

Of the 1196 included children, the pubertal stages were obtained in a subset of 747 cases (495 girls, 252 boys). When using an adjusted GLM model to analyze the relationship between Tanner stages and leptin, sOB-R, and FLI, there was no continuous relationship with sOB-R in neither girls nor boys ($p = 0.363$ and $p = 0.178$, respectively), whereas both leptin ($p < 0.0001$ and $p = 0.0003$, respectively) and FLI ($p < 0.0001$ and $p = 0.0043$, respectively) was significantly correlated to Tanner stages.

For FLI, we performed a selective analysis of levels of the individual pubertal stages in girls and boys. We found significantly elevated sOB-R concentrations in Tanner stage I in girls ($p < 0.0001$) but no difference in boys ($p > 0.05$), when comparing to Tanner stage V by Wilcoxon-Mann-Whitney tests.

Discussion

Pediatric reference intervals

Many laboratory variables in pediatrics vary with sex, age, growth, and development and consequently the reference values for a given variable should ideally be established in the pediatric age ranges for both sexes. Various methods for establishing reference intervals have been proposed [39]. However, practical and ethical considerations often preclude the blood sampling in healthy children, which reduces the possibility of collecting large samples from a standard population of 'normal' children. Accordingly, the data on normative values for leptin in children are mainly based on smaller studies [24,25]. It is well-known that preanalytical, analytical, and postanalytical sources of variation can significantly affect the measurement of leptin concentration in the blood which makes direct

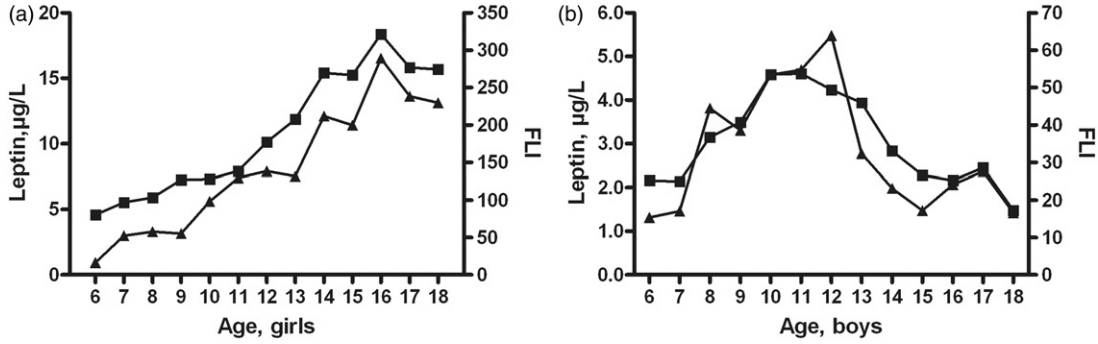

Figure 2. (a) Relationship between leptin and FLI in girls. Empiric values for 50th percentile for leptin (squares) and FLI (triangles) for each age group. (b) Relationship between leptin and FLI in boys. Empiric values for 50th percentile for leptin (squares) and FLI (triangles) for each age group.

Table 3. Percentiles of FLI calculated with GAMLSS.

	Percentiles for girls							Percentiles for boys						
Age (years)	2.5	5	25	50	75	95	97.5	2.5	5	25	50	75	95	97.5
6	0.71	1.38	8.31	24.23	63.32	216.45	312.36	0.27	0.63	5.10	16.45	45.21	157.36	226.35
7	0.82	1.63	10.23	30.59	81.56	285.40	414.55	0.37	0.86	6.95	22.40	61.58	214.61	308.87
8	0.96	1.93	12.69	38.90	105.93	380.37	556.64	0.51	1.16	9.34	30.10	82.75	288.79	415.86
9	1.11	2.29	15.80	49.67	138.20	509.11	750.49	0.67	1.52	12.22	39.34	108.17	377.97	544.58
10	1.26	2.66	19.50	63.05	179.01	672.91	996.80	0.85	1.94	15.53	49.96	137.41	480.76	693.09
11	1.31	2.91	23.44	78.31	226.36	858.91	1272.64	1.06	2.40	19.14	61.55	169.31	593.17	855.61
12	1.22	2.93	26.90	93.38	274.03	1038.02	1531.28	1.27	2.86	22.72	72.97	200.80	704.39	1016.62
13	0.99	2.68	29.58	107.35	318.93	1192.99	1744.72	1.45	3.26	25.75	82.65	227.50	799.10	1153.96
14	0.65	2.17	31.61	120.98	362.23	1319.13	1902.53	1.59	3.56	27.96	89.69	246.92	868.45	1254.82
15	0.34	1.56	33.38	135.57	405.86	1415.01	2001.55	1.69	3.78	29.56	94.74	260.91	918.87	1328.45
16	0.19	1.16	35.90	152.67	451.08	1484.50	2052.60	1.78	3.96	30.82	98.73	271.95	959.01	1387.25
17	0.17	1.08	40.05	172.14	493.91	1521.73	2055.71	1.85	4.12	31.86	101.98	280.98	992.14	1435.98
18	0.21	1.28	45.85	192.02	528.91	1525.15	2016.67	1.91	4.24	32.77	104.81	288.86	1021.25	1478.93

comparison of various studies difficult [25]; yet there is a need to establish reference values for leptin concentrations in children [24].

Our study

In this study, the serum concentrations of leptin and sOB-R in a large cohort of 1196 healthy, non-obese Danish schoolchildren were investigated. The data fills a gap in the literature, as normative data on serum leptin concentrations and particularly on sOB-R in children are sparse when compared to adults [24,25]. Furthermore, very little of the available data are derived from healthy, non-obese children and accordingly many conveniently available pediatric study materials that have been used as reference groups may occasionally harbor biases, that should be identified and adjusted. In contrast, the group of children that was examined in the present study is less likely to be biased as the children were unselected healthy schoolchildren.

Leptin in relation to previous publications

We demonstrated an age-dependent correlation with both serum leptin concentration and FLI with a gradual increase in girls throughout childhood and adolescence. In contrast, boys had significantly higher leptin concentration and FLI in early adolescence. Both these findings are in accordance with previously published data [19,24,40]; albeit our data did not study pre-school children which hinder direct comparison. Still, we demonstrated relatively high levels of leptin in boys at the onset of puberty which is in concordance with previous finding [19,41], and it is believed to reflect the influence of rapidly rising serum sex steroids concentration on leptin production [42].

Finally, the observed difference in leptin concentrations between our data and previous studies may reflect genetic and/or metabolic differences in the study populations. However, it may also reflect technical differences such as the known difficulties in leptin measurements [25] and it has long been stated that caution should be taken when comparing absolute leptin concentrations across studies [43]. These factors may also manifest themselves as increased variation and explain the relatively wide reference intervals that were observed, although we don't have data to support this.

sOB-R in relation to previous publications

We observed no difference in the sOB-R concentrations between sexes. Nor did we demonstrate an age-dependent correlation with sOB-R concentrations throughout the analyzed age span; however, we did observe significantly elevated sOB-R in the younger children, i.e. girls younger than 10 and boys younger than eight. These findings are also largely in concordance with previous findings [19,40], that demonstrated higher values in younger children, albeit up to 11 years in both sexes. In contrast to the sex-specific leptin pattern, the sOB-R concentrations were inversely correlated to age in both sexes and correspondingly we found the highest sOB-R concentrations in prepubertal children, i.e. Tanner stage I. It has been proposed that the high concentrations of

sOB-R in early childhood may at least in part suppress leptin actions on its membrane receptors [19], but this study does not provide sufficient data to elucidate this theory. We observed a correlation between FLI and leptin (Figure 2), which indicates that the plasma concentration of leptin in healthy, non-obese children is relatively independent of the sOB-R concentration.

Study strengths

Several variables are known to potentially influence leptin measurements [25]. Accordingly, care was taken to minimize the preanalytical [44] variables as all samples were taken in a relatively narrow time span and after an overnight fast which should reduce the influence from the circadian and ultracircadian leptin cycling [45–47] as well as the potential influence from leptins interactions with insulin [48].

As all measurements in this study were done in-house and in batch using the same method, the presented data can be considered valid regarding potential biases, i.e. variation in the analysis methods. The structured family-based questionnaire concerning general health data was self-reported and therefore subject to potential bias. However, it is relatively unlikely for any systematized bias to have influenced the data.

Study weakness

Several statistical methods exist for dealing with interpreting data which include values below LOD, including deletion of non-detects, single substitution, extrapolation by regression, and multiple imputation using TOBIT modeling. We applied the latter as it is believed to be more accurate [34,36]. However, regardless of statistical approach to dealing with values below LOD, data are per definition skewed. Therefore, the sOB-R values $<1.56\,\mu g/L$ must be interpreted with caution. Considering the relatively low percentage of non-detects ($<$ 10%) and the range of sOB-R concentration observed, it is reasonable to presume the influence is small. A potential weakness is pre analysis storage of samples. However, an analysis of the pre-analytic variability demonstrated stability of leptin and sOB-R in the blood sample. Given that the samples were stored at $-80\,^{\circ}C$ and only subjected to one freeze-thaw cycle no pre-analytic condition is likely to have influenced the results.

Conclusion

Hyperleptinaemia has been associated with increased risk of the metabolic syndrome and cardiovascular disease [20–22] and it has been proposed that alterations in plasma leptin concentrations and FLI may help evaluating phenotypically non-obese children by detecting those with more unfavorable risk profiles independent of BMI status [49]. The data presented in this study may therefore potentially help in metabolic risk stratification of children; a task that is likely to gain in importance in the near future as a consequence of the high prevalence of childhood obesity and its associated complications.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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